FORAGING ECOLOGY AND CONTAMINANT BURDENS OF RINGED SEALS (*Pusa hispida*) IN THE CENTRAL CANADIAN ARCTIC

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Abstract

Increasing atmospheric and ocean temperatures have led to dramatic declines in the thickness and extent of Arctic sea ice, resulting in a range of population-level effects on native wildlife, including shifts in distribution, diet, and exposure to environmental contaminants. Due to their reliance on sea ice habitat and central position within the Arctic marine food web, ringed seals (*Pusa hispida*) are particularly vulnerable to these climate-mediated changes. In this thesis, I sought to investigate factors affecting ringed seal foraging habits and contaminant burdens in the Gulf of Boothia and Prince Regent Inlet, located in the central Canadian Arctic. Specifically, I used fatty acid, stable isotope and stomach content analyses to examine how seal diets differed in accordance with demographic and environmental variables. Contaminant concentrations in blubber and muscle tissue were also assessed in relation to relative trophic position and broad-scale climate indices. Overall, diet analyses revealed intraspecific and temporal variation in ringed seal foraging. Changes in sea ice phenology also influenced ratios of stable carbon isotopes, which provide an indication of foraging habitat. Contaminant concentrations exhibited similar demographic variation and also differed in accordance with changes in the timing of sea ice breakup and freeze-up, as well as shifts in the Arctic and North Atlantic Oscillations. Ongoing monitoring of ringed seals in this region is important, as climate-mediated shifts in prey composition and contaminant levels may be indicators of broader ecological changes.

Dedicated to my father, Robert Douglas Ross.

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Chapter I – General Introduction

Arctic Marine Food Webs

Food webs describe the feeding relationships among species within an ecological community (Bersier *et al*., 2002). Organisms are categorized into relatively discrete trophic levels, which reflect the dynamics of energy transfer from primary producers to intermediary consumers and ultimately apex predators (Elton, 1927; Lindeman, 1942). Trophic interactions may be regulated by resource availability (bottom-up effects), predation (top-down effects), or some combination of the two, as the relative abundance of different species change over time and space (White, 1978; McQueen *et al.*, 1986; Leroux and Loreau, 2015). Despite low species richness, Arctic marine food webs are relatively long, spanning approximately five distinct trophic levels (Payer *et al.*, 2013; Hobson and Welch, 1992). In addition to polar cod (*Boreogadus saida*), which play a particularly important role in the transfer of energy from lower trophic organisms to numerous species of marine birds and mammals (Bradstreet and Cross, 1982), ringed seals (*Pusa hispida*) are widely considered a keystone species because of their central position in Arctic food webs (Ferguson *et al.*, 2005; Smith, 1987). They are an opportunistic trophic omnivore, preying on a variety of crustaceans (*e.g.* amphipods, mysids), and several species of both benthic and pelagic fishes (Yurkowski *et al.*, 2016; Holst *et al.*, 2001; Chambellant *et al.*, 2013). Ringed seals are also the most common prey for polar bears (*Ursus maritimus*) and continue to play an integral role in the diet of many Inuit and northern Indigenous people (Ramsay and Stirling, 1988; Reeves, 1998; Chambellant *et al.*, 2012). Accordingly, ringed seals act as a trophic link between numerous species near the base of the Arctic marine food web and several top predators.

Ringed Seal Ecology

General Overview

Ringed seals are distributed throughout the circumpolar Arctic and are the most abundant pinniped species in the northern polar regions, believed to have a global population of approximately three million (Lowry, 2016; Frost and Lowry, 1981). Considered a pagophilic, or 'ice loving' species, they are inexorably reliant on sea ice for several essential life history events (Yurkowski *et al.,* 2016; Riedman, 1990). Specifically, sea ice is the platform atop which seals breed, give birth and wean their pups. In the spring of each year, parturient females construct subnivean birth lairs in snowdrifts that form on landfast sea ice (Riedman, 1990; Reeves, 1998). These lairs are accessed from below, rendering them difficult to detect from the ice's surface, which is an important feature that helps protect their vulnerable newborn pups from inclement weather and predation. After giving birth in these lairs, pups are weaned from April to May (Reeves, 1998). This period of parental care is followed closely by a two-week breeding season and annual moult, all of which occur on remnant sea ice. Thus, the importance of sea ice to their long-term survival, certainly at current densities, cannot be overstated.

Foraging Ecology

Following the spring moult, which coincides with a period of fasting, ringed seals feed intensively during the open-water season in late summer and fall (Chambellant *et al.,* 2013; Young and Ferguson, 2014). They consume a large variety of prey, including invertebrates such amphipods, euphausiids, decapods and mysids, as well as numerous species of fish, including sandlance (*Ammodytes sp.*), sculpins (*Myoxocephalus sp.*), capelin (*Mallotus villosus*) and polar cod (Holst *et al.,* 2001; Chambellant *et al.*, 2013). Although several of these species are

common throughout most their range, ringed seal diet composition has been shown to vary according to season, life stage, sex, and geographical local (Bradstreet and Finley, 1983; Smith, 1987; Thiemann *et al.*, 2007; Holst *et al.*, 2001, Yurkowski *et al.*, 2016). Sea ice phenology also has a significant impact on the distribution of many of their primary prey species, which directly influences their foraging habits (Yurkowski *et al*., 2016). For instance, ringed seals inhabiting higher latitudes, where the ice-free season is shorter, have been shown to travel greater distances in search of preferred prey than those inhabiting lower latitudes where prey may be more readily available (Lowry *et al*., 1980; Yurkowski *et al.*, 2016).

Ringed seal foraging ecology has been studied using several diet estimation techniques, ranging from traditional methods, such as stomach content analysis, to more recent approaches, including stable isotope and fatty acid analyses (McLaren, 1958; Holst *et al.*, 2001; Young and Ferguson, 2013; Thiemann *et al.*, 2007). Stomach content analysis involves the recovery and examination of undigested prey hard parts from a predator's gastrointestinal tract in order to quantify the different proportions of species contained within the sample (Bowen and Iverson, 2013). One of the primary limitations of this approach is the fact that samples only represent a small subset of what each predator eats, generally the last one or two meals prior to being harvested. Also, some material may undergo complete or partial digestion, resulting in an underestimate of the true proportion of these species in the predator's diet (Bowen and Iverson, 2013). Furthermore, any soft-bodied prey items, including plant matter, will likely be completely overlooked when relying solely on this technique.

Stable isotope analysis is another fairly common technique used to study the diets and trophic position of Arctic marine mammals, including of ringed seals (Young and Ferguson, 2014; Ramsay and Hobson, 1991; Yurkowski *et al.*, 2016). Using ratios of nitrogen and carbon

isotopes ($\delta^{15}N$ and $\delta^{13}C$, respectively), one can glean important information about seal foraging habits because these isotopic signatures are passed, slightly modified, from prey to predator in fairly predictable ways. Nitrogen stable isotopes are subject to trophic enrichment, whereby $\delta^{15}N$ increases by approximately 3-5‰ from prey to predator, and therefore provides information concerning trophic level positioning (Young and Ferguson, 2014; Matley *et al.*, 2016). Carbon isotopes ($\delta^{13}C$) on the other hand are only slightly enriched with increasing trophic levels; however, they do provide a useful indicator of foraging habitat (*e.g.* inshore *vs*. offshore, benthic *vs.* pelagic; DeNiro and Epstein, 1978; Dunton, 1989).

Fatty acids (FA) can also serve as indicators of trophic relationships in marine food webs and have been used to study ringed seal diets throughout the Canada Arctic (Brockerhoff *et al.,* 1967; Iverson *et al.,* 1997; Thiemann *et al.,* 2007; Young and Ferguson, 2013). Because monogastric predators, such as ringed seals, are limited in their ability to synthesize FAs, dietary FAs are predictably incorporated into adipose stores with little or no modification (Budge *et al.*, 2006; Thiemann *et al.,* 2008). Collectively, these dietary FAs are referred to as an FA signature and reflect an individual's diet over several weeks to months (Kirsch *et al.,* 2000; Budge *et al.,* 2006). These FA signatures may then be used to examine intraspecific patterns and temporal trends in foraging habits (Iverson *et al.,* 1997; Thiemann, 2008).

Contaminant Burdens in Ringed Seals

Given their ubiquitous circumpolar distribution and central position within Arctic marine food webs, ringed seals are also commonly used as an indicator species to monitor spatial and temporal trends in both legacy and emerging contaminants (Vorkamp and Muir, 2016; Muir *et al.,* 1999). Research examining chemical concentrations in ringed seals dates back to the 1970s when concerns about exposure to persistent organic pollutants (POPs), including polychlorinated

biphenyls (PCBs) and organochlorine pesticides (OCPs) were high due to the prevalence of their use (Muir *et al.*, 1992). Many of these chemicals were produced in mid-latitude regions and used as flame retardants, hydraulic lubricants, pesticides, and in numerous other industrial applications (Tanabe, 1988; Muir *et al.*, 1999). Due to their toxicity, production of many of these chemicals was banned between the early 1980s and 2004; however, they continue to persist in ecosystems around the globe, including the Arctic, where they readily bio-magnify and accumulate in terrestrial and marine food webs (Muir and Howard, 2006; AMAP, 2018; Atwell *et al.*, 2008). Heavy metals released as by-products of fossil fuel combustion and other anthropogenic applications also accumulate in Arctic biota and the physical environment (Dietz *et al.*, 1998).

Concentrations of many of these compounds vary geographically, often along longitudinal and/or latitudinal gradients. These patterns arise due to differing source locations and unique physiochemical characteristics, which influence how each compound is transported and deposited into the environment (Houde *et al.*, 2019; Macdonald *et al.*, 2000; Braune *et al.*, 2005). Exposure rates in ringed seals generally reflect these spatial gradients, and while levels of some compounds have increased in recent years, overall concentrations in seals have declined over the last several decades due to the aforementioned ban on their production and use (Houde *et al.*, 2019). Information on the health effects associated with these compounds in ringed seals is sparse; however, previous studies have shown elevated concentrations of certain chemicals may result in reproductive impairment, depressed immune function, and skeletal deformities (Vorkamp and Muir, 2016; Routti *et al.*, 2019). Due to their trophic position, contaminant levels observed in ringed seals typically reflect those reported in polar bears and other common predators, as well as the numerous species upon which they prey. Therefore, quantifying

contaminant concentrations in ringed seals provides insights into the health of the broader Arctic marine community.

Climate Change

Currently, the Arctic is warming at a rate two to three times faster than the global average (IPCC, 2014; Serreze and Barry, 2011). Increasing atmospheric and ocean temperatures have severely affected the thickness and extent of Arctic sea ice (Comiso, 2002; Gagnon and Gough, 2005; Kwok and Rothrock, 2009), resulting in a range of effects on wildlife, including shifts in species distribution (MacCracken, 2012), diminished body condition (Stirling and Derocher, 2012), and population declines (Regehr *et al*., 2007). Because of their reliance on sea ice for several essential life history events, ringed seals are particularly vulnerable to climate-mediated changes in sea ice habitat (Laidre *et al.*, 2015). For instance, longer periods of open water have been shown to coincide with reduced rates of ovulation and pregnancy, as well as elevated levels of stress (blubber cortisol) in seals from Hudson Bay (Ferguson *et al.*, 2017). Higher spring temperatures and unusual rain events, which are expected to become increasingly common as the Arctic continues to warm, have been associated with soft snow conditions that lead to the premature collapse of ringed seal birth lairs (Bintanja and Andry, 2017; Stirling and Smith, 2004). The removal of protection offered by these subnivean lairs likely exposes newborn pups to harsh weather conditions and higher rates of predation, which may negatively affect overall recruitment and ringed seal population health (Stirling and Smith, 2004; Smith and Stirling, 1975).

Climate change has also influenced ringed seal diet in southern portions of the species' range. Chambellant *et al.* (2013) showed that the importance of polar cod declined significantly between the 1990s and mid-2000s when they were replaced by capelin, a common subarctic

species, as the primary prey recovered in dietary analyses. These observed changes in prey composition likely reflect shifts in the relative abundance of the two species, and were associated with increased ocean temperatures in Hudson Bay (Chambellant *et al.,* 2013; Gaston *et al.,* 2003). Further shifts in the distribution of ice-associated prey, and northward expansion of traditionally subarctic species are expected to continue in concert with warming Arctic temperatures (Tynan and DeMaster, 1997; Gaston *et al.,* 2003). While ringed seals do exhibit a high degree of dietary plasticity (see Yurkowski *et al.,* 2016), the energetic costs associated with these observed and projected changes remain poorly understood.

Warmer ocean temperatures and continued loss of sea ice are also projected to alter exposure pathways of both legacy and emerging contaminants (AMAP, 2017; McKinney *et al.,* 2015). Reductions in total sea ice extent, for example, may allow for greater direct absorption of gas-phase contaminants into ocean surface waters where they can be readily incorporated into the marine food web (Macdonald *et al.,* 2000). Previous studies have also documented associations between increased contaminant concentrations in various taxa and climate indices, including the Arctic Oscillation and North Atlantic Oscillation (Cabrerizo *et al.,* 2018; Houde *et al.,* 2019). Positive phases of these indices generally coincide with the northward movement of air from mid latitudes, likely facilitating the transportation of greater volumes of volatile chemicals to the Arctic (Houde *et al.,* 2019). Along with other top predators, ringed seals are particularly vulnerable to increased levels of environmental contaminants, as many of these chemicals are known to become increasingly concentrated in upper-trophic-level species (Hoekstra *et al.,* 2003; Atwell *et al.,* 2008).

Historical analyses of sea ice conditions in the Gulf of Boothia suggest large portions of the region contained primary ringed seal habitat (*e.g.* first-year pressure ridges, edges of mobile

ice; Barber and Iacozza, 2004). However, long-term trends in the timing of spring breakup and fall freeze-up show the total number of ice-covered days has decreased by 8.6 days decade $^{-1}$ since the 1970s (Stern and Laidre, 2016). Further, total Arctic sea ice extent in four successive winters (2015-2018) were the lowest on record, suggesting recent conditions may be less favourable (Overland *et al.*, 2018). Due to a paucity of information on ringed seals in this region and the non-uniform ways in which climate change is expected to affect Arctic marine communities, it is important to document baseline information against which future climate-mediated changes in habitat, prey availability, physiology and contaminant burdens may be assessed.

Thesis Objectives and Organization

Overall, the primary objectives of this research were to evaluate the foraging habits and contaminant burdens of ringed seals in the Gulf of Boothia, Nunavut. This region of the central Canadian Arctic supports the highest density of polar bears in the circumpolar North, yet little is known about their primary prey, the ringed seal (Taylor *et al.*, 2009; Hamilton and Derocher, 2019). Thus, I attempt to fill important knowledge gaps concerning their ecology and provide baseline information against which future changes in their diet and population health may be evaluated. This thesis is formatted as two independent manuscripts (Chapter II and Chapter III), along with a general introduction (Chapter I) and conclusion (Chapter IV).

Chapter II examines the feeding ecology of ringed seals from the Gulf of Boothia and Prince Regent Inlet using three complimentary diet estimation techniques (fatty acid signature, stable isotope, and stomach content analysis). Foraging habits are assessed in relation to several demographic and environmental variables, including sex and age class, as well as sea ice

phenology (annual date of sea ice breakup and freeze-up during the year of sample collection and the preceding year).

Chapter III examines concentrations of legacy contaminants in ringed seals from the Gulf of Boothia and Prince Regent Inlet. Persistent organic pollutants (POPs), including DDT and PCBs, along with a suite of trace elements are examined in different tissues to assess how intrinsic and extrinsic factors may influence their accumulation in seals from this region of the Canadian Arctic. Contaminant concentrations are evaluated in relation to sex and age class, as well as sea ice concentration and type (first-year *vs.* multi-year ice), and mean annual values of the Arctic Oscillation and North Atlantic Oscillation climate indices.

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Chapter II – Complementary Diet Analyses Reveal Demographic and Temporal Variation in Ringed Seal (*Pusa hispida*) Foraging Habits in the Central Canadian Arctic

Introduction

Increasing atmospheric and ocean temperatures have precipitated significant declines in both perennial and seasonal Arctic sea ice cover over successive decades since the 1970s (Comiso *et al.*, 2008; Comiso, 2002; IPCC, 2014; Kwok and Rothrock, 2009). Decreases in ice extent and thickness have been non-uniform, yet nearly all regions of the circumpolar Arctic have experienced some degree of deterioration in long-term sea ice conditions (Xia *et al.*, 2014; Tynan and DeMaster, 1997). The Gulf of Boothia, located in the central Canadian Arctic, is among those areas most impacted by climate-driven sea ice changes. The timing of spring breakup now occurs significantly earlier than it did forty years ago, retreating at a rate of approximately 8.6 days per decade^{-1}. Combined with later fall freeze-up, the number of ice-covered days has also decreased by roughly 20 days per decade^{-1}, resulting in a considerably longer open water season (Stern and Laidre, 2016).

These changes in sea ice phenology have had widespread negative effects on Arctic marine food webs (Kortsch *et al.*, 2015; Post *et al.*, 2013; Laidre *et al.*, 2008). Shifts in the timing of spring breakup have caused mismatches in resource availability and the energy requirements of primary consumers. This, in turn, has stemmed the transfer of energy and biomass to higher trophic levels, likely affecting the body condition, fecundity and survival of larger consumers (Post *et al.*, 2013; Tynan and DeMaster, 1997; Kovacs *et al.*, 2011). Sea ice loss also directly impacts ice-obligate species through the degradation of critical habitat and changes in prey availability (Derocher *et al.*, 2004; Ferguson *et al.*, 2017; Gaston *et al.*, 2003).

Evidence of these climate-mediated impacts have been observed in ringed seals in Hudson Bay and the western Arctic, where they have exhibited ongoing declines in body condition and increased physiological stress over several decades in relation to progressively longer open water periods (Harwood *et al*., 2012; Ferguson *et al.*, 2017). Continued declines in sea ice are projected to negatively affect ringed seal demography (Reimer *et al*., 2019).

Ringed seals are relatively small marine carnivores distributed throughout the circumpolar Arctic (McLaren, 1993; Smith *et al.*, 1991; Reidman, 1990). Considered a pagophilic, or 'ice loving' species, they are reliant on sea ice for mating and parturition, which occurs in subnivean birth layers atop the ice during early spring (Yurkowski *et al.*, 2016; Smith and Stirling, 1975; Riedman, 1990). They feed opportunistically and have been shown to undergo an ontogenetic shift in foraging habits, transitioning from a diet comprised mostly of invertebrates to fish (Holst *et al.*, 2001; Young and Ferguson, 2010; Yurkowski *et al.*, 2016). Overall, they prey on a variety of crustaceans, including amphipods, mysids and decapods; adult and juvenile seals also routinely prey on small- to medium-sized fishes, including sandlance (*Ammodytes sp.*), sculpins (*Myoxocephalus sp.*), and polar cod (*Boreogadus saida*) (Young and Ferguson, 2014; Reeves, 1998; Holst, *et al.*, 2001). Ringed seals also serve as an important prey species for other marine predators. Seals of all ages are regularly preyed upon by polar bears (*Ursus maritimus)* and killer whales (*Orcinus orca*; Higdon *et al*., 2011; Pilfold *et al*., 2012), while recently weaned pups are also vulnerable to opportunistic terrestrial and avian predators, such as foxes (*Vulpes sp*.), wolves (*Canis lupus*), and ravens (*Corvus corax*) (Reeves, 1998; Smith, 1980). Given their central position in the food web, seal foraging behaviour may provide important insights into predator-prey interactions influencing the structure and functioning of

Arctic marine communities. Further, changes in their foraging habits may reflect shifts in the distribution and abundance of prey and/or long-term shifts in environmental conditions.

Ringed seal foraging ecology has been studied using a variety of diet estimation techniques, ranging from conventional methods, such as stomach content analysis, to more recent approaches, including stable isotope and fatty acid analyses (McLaren, 1958; Holst *et al.*, 2001; Young and Ferguson, 2013; Thiemann *et al.*, 2007). Stomach content analysis involves the recovery of undigested prey hard parts from a predator's gastrointestinal tract. Provided there is an adequate sample size, this analysis allows one to make inferences about a population's feeding habits in terms of both prey composition and relative proportions (Bowen and Iverson, 2012). One of the primary limitations of this technique, however, is the fact that samples only represent a small subset of a predator's diet - generally the last one or two meals. Also, some material, such as otoliths, cephalopod beaks and any soft-bodied prey items may undergo partial or complete digestion, which results in an underestimate of the true proportion of these species in a predator's diet (Murie and Lavigne, 1986; Bowen and Iverson, 2012).

Given these limitations, recent studies examining ringed seal foraging ecology have also incorporated the use of stable isotope analysis (Holst *et al.*, 2001; Chambellant *et al.*, 2013; Young and Ferguson, 2014). Ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are assimilated into consumer tissues in a predictable manner and offer a time-integrated estimate of diet over longer timescales. For example, stable isotopes in muscle tissue reflect a diet incorporated over several weeks to months, while isotopes in metabolically inert tissues, such as teeth and claws, may provide an estimate of diet over a lifetime (Tieszan *et al.*, 1983; Hobson and Clark, 1992; Newsome *et al.*, 2010). In marine environments, nitrogen stable isotopes can be used to identify an organism's trophic position, as there is a relatively predictable increase in $\delta^{15}N$ values from

prey to predator (Kelly, 2000; Newsome *et al.*, 2010). In contrast, ratios of carbon remain relatively stable across trophic levels, but can provide useful information on foraging habitats (e.g. inshore vs offshore, benthic vs. pelagic; DeNiro and Epstein, 1978; Dunton, 1989).

Fatty acids (FA) can also serve as indicators of trophic relationships in marine food webs and have been used to study ringed seal diets throughout the Canada Arctic (Brockerhoff *et al.*, 1967; Iverson *et al.*, 1997; Thiemann *et al.*, 2007; Young and Ferguson, 2013). Because monogastric predators, including seals and other marine carnivores, are limited in their ability to synthesize FAs, dietary FA are predictably incorporated into adipose stores with little or no modification (Budge *et al.*, 2006; Thiemann *et al.*, 2008). Collectively, these dietary FAs are referred to as an FA signature and reflect an individual's foraging habits over several weeks to months (Kirsch *et al.*, 2000; Budge *et al.*, 2006). Due to the high degree of interspecific variation in FA signatures, proportional contributions of prey species to a predator's diet may be determined using a statistical model (see Iverson *et al.*, 2004). However, in the absence of a database detailing the FA composition of all potential prey, predator FA signatures alone can be used to examine intraspecific patterns and temporal trends in foraging habits (Iverson *et al.*, 1997; Thiemann, 2008).

In this study, I examined the foraging patterns of ringed seals in the Gulf of Boothia and Prince Regent Inlet between 2012 and 2016, using three complementary estimation techniques: fatty acid signature analysis, stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ ratios in muscle tissue, and stomach content analysis. I sought to (1) establish a baseline for ringed seal diets against which comparisons could be made in future studies, (2) evaluate age-related variability, and (3) evaluate interannual variability in ringed seal diets over time in relation to changes in the sea ice phenology.

Methods

Sample Collection

Evaluation of ringed seal foraging ecology in the Gulf of Boothia and Prince Regent Inlet was based on samples collected by Inuit hunters during ongoing subsistence harvests in Kugaaruk, Nunavut (Figure 2.1). From May 2012 to October 2016, hunters collected samples of blubber and muscle from 177 ringed seals (Table 2.1). Seals were harvested between May and September of each year. After collection, tissue samples were frozen at -20 °C and subsequently shipped to the Department of Fisheries and Oceans Freshwater Institute (Winnipeg, MB) where they were stored at -35 °C prior to laboratory analyses. Typically, the sex of each seal was determined in the field at the time of sampling. The age of each seal was determined by Matson Laboratory (Manhattan, MT) using the methods outlined in Stirling *et al.* (1977) and Stewart *et al.* (1996). Briefly, canine teeth were extracted from the lower jaw, decalcified, stained and longitudinally thin sectioned. Using a compound microscope and transmitted light, the number of annual growth layer groups (GLG) in each tooth was counted.

Based on initial age determination, seals were then separated into one of three age classes. Seals born in the spring and harvested during the same year were referred to as young of the year (< 1 yr); seals aged 1 to 5 were considered juveniles; and seals aged 6 years and older were considered adults (Holst *et al.,* 1999; Young and Ferguson, 2014).

Laboratory Analyses

A subsample of blubber, weighing approximately 0.5 g, was taken from the interior of each sample in order to avoid superficial tissue that may have undergone oxidation during storage (Budge *et al*., 2006). Following the methods outlined in Iverson *et al*. (2001), lipid was

quantitatively extracted and isolated using a modified Folch procedure (Folch *et al*., 1957). Fatty acid methyl esters (FAME) were derived from the extracted lipid using sulfuric acid as a catalyst (Thiemann *et al*., 2004; Budge *et al*., 2006). Duplicate analyses and identification of the resultant FAME were performed using a temperature-programmed gas chromatograph (GC) with a flame ionization detector fitted with a polar column (30 m x 0.25 mm inner diameter; DB-23, Agilent Technologies, Palo Alto, California, USA; Budge *et al*., 2006). FA are expressed as the mass percent of total FA ± SE, and referred to by the shorthand nomenclature of A:Bn-X, where A indicates the length of the carbon chain, B is the number of double bonds, and X is the location of the first double bond relative to the terminal methyl group (IUPAC-IUB, 1967). GC peaks corresponding to individual FA were verified and integrated using CompassCDS software (Version 3.0, Bruker Daltonics Inc., Germany) and manually corrected for those samples where retention times had shifted.

Muscle samples collected from 46 seals (Table 2.1) were freeze-dried for approximately 48 h, then crushed into a fine powder and homogenized. Lipids were extracted with 2:1 chloroform:methanol using a modified Bligh and Dyer (1959) procedure (Hobson *et al*., 1997; McMeans *et al*., 2009). Subsamples of the dried muscle homogenate were weighed to approximately 500 µg and placed into tin capsules for analysis. Stable-nitrogen and stable-carbon isotope values for each sample were determined using a Thermo Finnigan-Deltaplus (Thermo Finnigan, USA) mass spectrometer in conjunction with a Costech elemental analyzer (Costech, USA) at the University of Waterloo's Environmental Isotope Laboratory. Analytic precision was determined to be ± 0.2‰ and ± 0.3‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Accuracy was established using repeat analyses of internal laboratory standards calibrated against International Atomic Energy Agency Standards CH6 for carbon and N1 and N2 for nitrogen. Precision was assessed

by analysing 15% of samples in duplicate, yielding a within-sample coefficient of variation of 0.014 for $\delta^{15}N$ and 0.017 for $\delta^{13}C$. Stable isotope ratios are expressed in parts per thousand (‰) using delta (δ) notation and are calculated using the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \cdot 1{,}000$$

where *X* is ^{15}N or ^{13}C, and *R* is equal to the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample and standard material. The recognized standards for stable nitrogen and carbon isotope analyses are atmospheric nitrogen and Pee Dee belemnite limestone, respectively.

Ringed seal stomachs collected in 2012 (n = 17; Table 2.1) were thawed and weighed to the nearest 0.1 g using a Denver Instruments S1-4002 balance (Denver Instruments, USA). The contents of each stomach were emptied into a plastic tray, weighed (g wet weight), and washed through a series of graduated sieves (300-125 μm). Prey items, including large or intact organisms, sagittal otoliths and invertebrate remains were counted, weighed and identified to the lowest taxonomical level possible using a variety of reference materials (Campana, 2004; Klekowski and Węslawski, 1991, Keast and Lawrence, 1990). The number of fish present in each stomach was estimated by dividing the total number of otoliths recovered for each prey species by two. Foraging indices based on prey number and weight were used to estimate the relative importance of each species to ringed seal diet:

$$(FO_i) = (f_i/f_t) \cdot 100$$

where FO_i is the frequency of occurrence of each food category *i*, f_i is the number of stomachs containing prey species *i*, and f_t is the total number of stomachs containing food; and,

$$N_i = (n_i/n_t) \cdot 100$$

where N_i is the relative frequency of occurrence by number of each food category *i* in all of the stomachs containing food, n_i is the number of individuals of species *i*, and n_t is the total number of all prey species. Statistical comparisons of feeding habits among ringed seals based on stomach contents alone were not considered due to small sample sizes and limited temporal coverage.

Sea Ice Data

Daily sea ice concentration data were obtained from Nibmus-7 SMMR and DMSP SSM/I-SSMIS Passive Microwave Data (Cavalieri *et al*., 1996) available from the National Snow and Ice Data Centre (NSIDC) in Boulder, CO. Raster images of sea ice concentration throughout the circumpolar Arctic are provided in a polar stereographic projection with a minimum resolution, or grid cell size, of 25 x 25 km. Sea ice concentration values, expressed as a percentage, are accurate within ± 5% and ± 15% of the actual sea ice concentration during the winter and summer, respectively (Stern and Laidre, 2016). Sea ice area for a given region is defined as the *fractional sea ice concentration x grid cell area* summed over cells with sea ice concentration greater than 15%, which is the common threshold standard for accurately identifying the presence of sea ice (Parkinson, 2014; NSIDC, 2018).

Using methods similar to those detailed in Stern and Laidre (2016), daily sea ice concentration was calculated for the Gulf of Boothia and Prince Regent Inlet from 2012 to 2016. Given that Arctic sea ice typically reaches its maximum extent in March and its minimum extent in September, the mean sea ice area was calculated for both March and September over the period 2006-2016. The halfway point between these two values was chosen as the transition threshold, which was then used to determine the dates of annual sea ice retreat (breakup) and advance (freeze-up) for the duration of the study period. Specifically, the date of sea ice retreat

was defined as the date on which the sea ice area fell below the threshold value, while the date of fall sea ice advance was defined as the date on which the sea ice area surpassed the threshold value on its way to the winter maximum (Ferguson *et al.*, 2017; Young and Ferguson, 2014). The duration of the open water season is simply the number of days between the dates of spring sea ice advance and fall retreat. All analyses of sea ice concentration data were performed in ArcMAP 10.6.1 (ESRI, 2018).

Statistical Analyses

While individual FA signatures are known to be independent, the analysis of FA data poses two problems in terms of violating the assumptions of most multivariate techniques: firstly, the covariance matrices are almost always heterogeneous, and secondly, the data are never multivariate normal (Budge *et al.*, 2006). In order to account for these violations, each analysis included more samples in a group than the number of fatty acids examined (at least 5:1), and all of the data were first transformed using the following log transformation:

$$x_{trans} = \ln(x_i/c_i)$$

where x_i is a given fatty acid expressed as a percent of total fatty acids, x_{trans} is the transformed fatty acid data and c_i is the percentage of a reference fatty acid (Budge *et al.*, 2006). I used 18:0 as the reference fatty acid because it is thought to convey little information about diet and has been reliably used as a reference in other publications (Budge *et al.*, 2006, Galicia *et al.*, 2015).

Thirty of the most abundant and/or variable dietary and extended dietary FAs were examined using discriminant function analysis and multivariate analysis of variance (MANOVA) to assess differences in FA profiles among ringed seals from different age classes (Thiemann *et al.*, 2007; Iverson *et al.*, 2004). Classification rates based on both conventional and jackknife

procedures were used to assess the ability of discriminant function analysis to accurately assign seals to the correct group based on their FA signature. Due to the limited number of samples collected from certain demographic groups (e.g. juvenile males; n = 6), and the inclusion of FA data from seals that were not sexed at the time of sample collection, one-way MANOVA was used to test for differences among age classes without controlling for other factors. A subset of fifteen FAs (Table 2.1) was used in further analyses that included only adult seals of known sex (n = 84). Specifically, sex-related differences were tested using one-way MANOVA and interannual variation in FA signatures was assessed in relation to changes in the date of annual sea ice breakup and freeze-up during the year of sample collection as well as the preceding year using principal component analysis (PCA). Simple linear regression and Spearman's rank correlation were then used to investigate the relationship between *z*-score values derived from the first principal component and the ordinal dates, considered as a continuous and rank-ordered variable, respectively (McKinney *et al.,* 2009).

Prior to analysis, stable isotope data were assessed for normality and homogeneity of variance using the Shapiro-Wilk test for multivariate data and visual inspection of residuals. Results indicated that $\delta^{15}N$ data were normally distributed while $\delta^{13}C$ were not; accordingly, all data were log-transformed [log(-x) for $\delta^{13}C$ values] to better meet model assumptions. Two-way analysis of variance (ANOVA) was used to assess the effects of sex and age class on $\delta^{15}N$ and $\delta^{13}C$ values, while analysis of covariance (ANCOVA) was used to assess relationships between ordinal dates of break-up and freeze during the year of sample collection, as well as the preceding year, and stable isotope ratios.

Possible ontogenetic differences in dietary niches were further assessed by examining isotopic niche widths of the three age classes using Stable Isotope Bayesian Ellipses in R

(SIBER; Jackson *et al.*, 2011). Niche widths for each age class were calculated using standard ellipse areas (SEA) with a correction factor for small samples sizes (SEAc). The shape, size and position of each ellipse are defined by the $\delta^{15}N$ x $\delta^{13}C$ covariance matrix and posterior estimates of these parameters derived from Monte Carlo simulations (Jackson *et al.*, 2011). Resultant SEAc values can be compared to determine the degree to which isotopic niches of each group overlapped (Jackson *et al.*, 2011). Isotopic niche overlap is a number ranging between 0 and 1, representing the degree of overlap between the SEAs of two groups and provides an indication of foraging similarity (Young and Ferguson, 2014). Values approaching 1 represent considerable niche overlap, while values approaching 0 suggest divergent isotopic niches.

Unless otherwise noted, the significance of statistical tests was assessed at $\alpha = 0.05$ and all analyses were conducted in R version 3.4.4 (R Core Team, 2013).

Results

A total of sixty-five fatty acids were consistently identified in each ringed seal blubber sample. Average mass percentage values ranged from 0.012 ± 0.001% (22:2n-9) to 20.086 ± 0.249% (16:1n-7), and forty FAs averaged > 0.1 % across the three age classes over the course of the study period (Table 2.2). The thirty FAs used to assess differences in foraging habits among seals of different age classes accounted for 85% of total FAs identified, while the subset of 15 FAs used to evaluate sex-specific differences and interannual variation among adult seals accounted for 81%. The remaining FAs exhibited little variation, and individually accounted for ≤0.09% (range: 0.007-0.097%) of total FAs.

Demographic Variation

To examine possible ontogenetic differences in foraging habits among ringed seals in the Gulf of Boothia and Prince Regent Inlet, discriminant function analysis and one-way MANOVA were used to test whether FA signatures varied according to age class. Discriminant function analyses (Figure 2.2) assigned 85.6% and 73.8% of ringed seals to the correct age class using conventional and jackknifed classification procedures, respectively. Juvenile seals had the highest misclassification rate, while young of the year were consistently assigned to the correct group with > 90% accuracy. Overall, results of the one-way MANOVA indicated that FA signatures differed significantly among ringed seals of different age classes (Wilk's $\lambda = 0.190$, $F_{(2, 157)} = 5.764$, $p = < 0.001$); twenty-two of the thirty FAs tested exhibited significant age-specific variation (Figure 2.3). *Post hoc* tests examining pairwise differences in individual FAs revealed that twelve of the thirty FAs tested using discriminant function analysis and MANOVA varied significantly between adults and both juveniles and young of the year, while thirteen differed significantly between juveniles and young of the year (Table 2.3)

After controlling for the effect of sex, age class did not significantly influence stable isotope ratios of muscle $\delta^{15}N$ (ANOVA: $F_{(3, 42)} = 0.8508$, $p = 0.474$). However, on average, young of the year had the highest $\delta^{15}N$ values, followed by adults then juveniles (Table 2.4). Ratios of muscle $\delta^{13}C$ also did not differ significantly among age classes (ANOVA: $F_{(3, 42)} = 2.227$, $p = 0.060$).

Standard ellipse area with a correction for small sample size (SEAc) was calculated for each age class using muscle $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios pooled over the duration of the study period (Figure 2.4). The three age classes differed in their respective SEAc with young of the year exhibiting the largest isotopic niche and juvenile seals the smallest (YOY = 3.69‰^{2}; adult = 2.08‰^{2}; juvenile = 1.22‰^{2}). The degree of overlap among the three age classes,

determined by the extent to which their SEAc overlapped, ranged between 0.22 for young of the year and juveniles, and 0.44 for young of the year and adults. Niche overlap between juvenile and adult seals was 0.33.

Effects of Sea Ice Phenology on Foraging

Variation in adult FA signatures was assessed in relation to changes in the annual dates of sea ice breakup and freeze-up using simple linear regression with *z*-score values derived from PCA. Due to the limited number of years of available data, Spearman's rank correlation was also used to test associations between adult seal FA profiles and the ordinal dates considered as rank-ordered explanatory variables. With the exceptions of 20:1n-7 and 20:1n-9, fatty acid signatures did not differ by sex among adult seals (MANOVA: Wilk's $\lambda = 0.677$, $F_{(1, 82)} = 0.963$, $p = 0.529$); therefore, data from both sexes were pooled for principal component analysis. The first two principal components accounted for 85% of the total variation in FAs among adult seals (PC1: 68%; PC2: 17%), yet there was no significant relationship between FA signatures and the annual date of breakup during the year of sample collection (PC1: $R^2 = 0.005$, $F_{(1, 82)} = 0.445$, $p = 0.507$), or during the preceding year (PC1: $R^2 = 0.015$, $F_{(1, 82)} = 1.265$, $p = 0.264$). Similar, non-significant relationships were found between FA signatures and the dates of freeze-up during both the present (PC1: $R^2 = 0.025$, $F_{(1, 82)} = 2.060$, $p = 0.155$), and previous year (PC1: $R^2 = 0.046$, $F_{(1, 82)} = 4.094$, $p = 0.050$). Results of the Spearman's rank correlation test supported these findings, suggesting variation in sea ice phenology alone did not lead to changes in FA signatures among adult seals. FA signatures of adult ringed seals did, however, exhibit significant interannual variation throughout the five-year study period (MANOVA: Wilk's $\lambda = 0.005$, $F_{(4, 79)} = 12.256$, $p >> 0.05$; Figure 2.5); eight of the fifteen FAs tested varied among years (16.2n-4, 18.1n-9, 18.2n-6, 20.1n-11, 20.1n-7, 20.5n-3, 22.1n-11, 22.6n-3.

Stable carbon isotope ratios varied in accordance with changes in sea ice phenology over the duration of the study. Specifically, after controlling for the effect of sex, the annual date of sea ice retreat had a significant effect on muscle $\delta^{13}C$ ratios in adult ringed seals (ANCOVA: $F_{(2, 32)} = 9.084$, $p = < 0.001$), as did the dates of freeze-up during the year of sample collection (ANCOVA: $F_{(2, 32)} = 12.340$, $p = < 0.001$), and the preceding year (ANCOVA: $F_{(2, 32)} = 8.381$, $p = 0.001$). Conversely, sea ice phenology did not appear to affect $\delta^{15}N$ ratios. Figure 2.6 shows $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios in both adult male and female ringed seal muscle relative to annual changes in the date of sea ice retreat. Carbon stable isotope ratios exhibit an inverse relationship with the date of sea ice retreat, whereby annual breakup dates that occur later in the spring/summer season correspond with lower mean $\delta^{13}C$ values. Mean annual $\delta^{15}N$ ratios varied little over the duration of the five-year study period.

Stomach Content Analysis

Sixteen of the seventeen ringed seal stomachs contained some dietary contents other than stones and parasites, which were found in 12% and 29% of the stomachs, respectively. The remaining stomach contained a single helminth parasite, but no observable food remains. In total, at least eleven different prey items were identified, including five species/genera of fish (polar cod, capelin [*Mallotus villosus*], sculpin, sandlance and snailfish [*Liparis sp.*), three amphipod species (*Themisto libellula, Gammarus wilkitzkii* and *Onisimus glacialis*), one decapod species (*Lebbeus polaris)* and one mysid (*Mysis oculata).* Among adult seals, ingested prey items consisted predominantly of fish, particularly polar cod and sculpins (Table 2.5). Young of the year also consumed relatively high proportions of polar cod; however, mysids comprised the bulk of their dietary intake. Adults and young of the year also fed on several amphipod species (*Themisto libellula* and *Gammarus wilkitzkii*), while adults were the only age class to consume

the decapod species *Lebbeus polaris.* The dietary items found in juvenile seal stomachs consisted exclusively of fish remains from polar cod and sculpin.

Discussion

The foraging habits of ringed seals have been assessed across broad spatial scales throughout the Canadian Arctic using a variety of diet estimation techniques (Smith, 1987; Chambellant *et al.*, 2013; Thiemann *et al.*, 2007). Notwithstanding general similarities in common prey items, previous studies have documented considerable regional differences among dietary tracers, including fatty acid signatures and stable isotope ratios (Thiemann *et al., 2007*; Young and Ferguson, 2014; Holst *et al.*, 2001). This variability is likely attributable to disparate prey assemblages and the localized foraging habits of ringed seals (Bluhm and Gradinger, 2008; McLaren, 1958). Given this regional variability and the non-uniform way in which climate change is expected to affect prey availability throughout the Arctic (Tynan and DeMaster, 1997), it is important to document local foraging habits of ringed seals to monitor fine-scale changes in habitat and overall population health. This study is the first to document the dietary habits of ringed seals in the Gulf of Boothia and Prince Regent Inlet, Nunavut, thereby providing novel insights into the structure and functioning of a central Arctic marine food web experiencing long-term environmental changes.

Intraspecific Variability

The FA composition of marine mammal blubber is largely determined by diet and has been shown to vary among different age classes in several pinniped species (Newland *et al.,* 2009; Beck *et al.,* 2004; Thiemann *et al.,* 2007). Here, I found considerable age-specific variation in the FA signatures of ringed seals in the Gulf of Boothia and Prince Regent Inlet.

Twenty-two of the thirty FAs included in analyses differed between age groups, with a total of twelve FAs differing between adults and both juveniles and young of the year. These findings are consistent with those presented in Thiemann *et al.* (2007), where the FA signatures of ringed seals harvested at several locations throughout the Canadian Arctic (Frobisher Bay, Jones Sound, Labrador Coast) varied by age, particularly between adult and juvenile seals. Considering FA signatures have been shown to mirror fine-scale differences in local prey assemblages (see Iverson *et al.*, 1997), these trends may be a reflection of ontogenetic resource partitioning arising from the spatial segregation of age classes. For example, juvenile seals are commonly excluded from fast ice habitats and forced to forage further offshore during the winter and spring months (McLaren, 1958; Smith, 1973; Krafft *et al.*, 2007). Adults and young of the year on the other hand often remain in near-shore areas for most of the year, venturing into pelagic habitats to forage only during the open water season (Smith, 1973; Luque *et al.*, 2014). Adults have also been documented diving to greater depths and for longer durations, ostensibly to forage on demersal prey that may be unavailable to immature seals (Harwood *et al*, 2015; Born *et al.*, 2004).

Although small sample sizes precluded statistical comparisons of stomach contents among age classes in the present study, previous research in the western Arctic and northern Baffin Bay showed adult seals fed predominantly on polar cod, whereas the diet of immature seals consisted primarily of pelagic amphipods (Lowry *et al.*, 1980; Smith, 1987; Holst *et al.*, 2001). While these species are often associated with near- and undersurface sea ice habitats (Tynan and DeMaster, 1997; Bradstreet and Cross, 1982; Gardinger and Bluhm, 2004), Majewski *et al.* (2015) found that polar cod biomass and mean length were positively correlated with increasing depth in the Beaufort Sea, reaching peak values at 350-500 m and 200 m,

respectively. These results support the notion that adult seals, which have been recorded diving to depths of 200-500 m (Harwood *et al.*, 2015), may be able to access and consume larger proportions of polar cod than immature individuals that tend to remain in near-surface waters (≤ 50 m) where amphipods are most abundant (Born *et al.*, 2004). Further, juvenile seals were significantly enriched in the FA 22:1n-11 relative to the two remaining age classes. Previous studies have demonstrated that this particular FA is often associated with pelagic fish (Budge *et al.*, 2002; Anderson *et al.*, 2004). Thus, combined with likely differences in foraging experience (Holst *et al.*, 2001; Chambellant *et al.*, 2013), the non-uniform distribution and utilization of prey may account for the observed age-specific variation in FA signatures among ringed seals in the Gulf of Boothia and Prince Regent Inlet.

Stable isotope analysis revealed a non-significant relationship between age class and $\delta^{15}N$ ratios in ringed seals; however, when examining group means, young of the year were enriched in $\delta^{15}N$ relative to the older age classes. This is likely a consequence of the direct transfer and assimilation of maternal isotopes into yearling muscle tissue during the nursing period when newborns feed exclusively on milk (Hobson *et al.*, 1997; Holst *et al.*, 2001). McLaren (1958) showed that stomachs of newborn seals harvested in the spring contained only curdled milk, whereas seals collected after mid-June had consumed milk, a variety of marine crustaceans, or a combination of the two. These observations, along with results presented in previous studies, suggest young of the year initially occupy a seemingly higher trophic position, but undergo a reduction in muscle $\delta^{15}N$ as they transition from a diet of milk to one consisting primarily of pelagic amphipods during the late summer and fall (Holst *et al.*, 2001; Young and Ferguson, 2014). The higher $\delta^{15}N$ values observed in adults, however, likely reflect a long-term diet

consisting of prey occupying upper trophic levels, including polar cod and other fishes (Holst *et al.,* 2001; Young and Ferguson, 2014; Chambellant *et al.,* 2013).

Carbon isotope ratios did not vary significantly among age classes. Similarly, analysis of age-specific niche widths revealed considerable overlap in isotopic signatures, particularly among adults and young of the year. As noted above, this is likely due to the direct transfer of maternal isotopes to newborn seals via milk; however, these results are at odds with those obtained in previous studies (Young and Ferguson, 2014; Holst *et al.*, 2001; Chambellant *et al.*, 2013; Young *et al.*, 2010). For example, Young and Ferguson (2014) observed differences in $\delta^{13}C$ ratios among all three age classes, and showed isotopic niche widths of adults differed considerably from pups in both eastern and western Hudson Bay. In contrast, Holst *et al.* (2001) found ^{13}C ratios only varied among adult and juvenile seals harvested in Grise Fiord and Qannaq, where stomach content analysis revealed juveniles consumed higher proportions of the pelagic amphipod *T. libellula.* Although not statistically significant, the higher carbon isotope ratios observed in adult seals from the Gulf of Boothia and Prince Regent Inlet may suggest adults do in fact consume higher proportions of benthic or demersal prey, as they are known to be enriched in $\delta^{13}C$ relative to pelagic species (DeNiro and Epstein, 1978; Dunton, 1989).

Temporal Variation

Consistent with previous findings, sea ice conditions in the Gulf of Boothia exhibited considerable interannual variability (Barber and Iacozza, 2004). Between 2012 and 2016, the annual date of spring breakup differed by as much as twenty days, but showed no clear short-term trend, neither increasing nor decreasing over the five-year study period. The FA signatures of adult seals also exhibited significant interannual variation; however, they did not change in relation to the dates of breakup or freeze-up, suggesting sea ice phenology alone does not

regulate ringed seal foraging habits. Rather, the observed year-to-year variation is likely attributable to natural fluctuations in prey abundance and distribution caused by complex interactions between several extrinsic factors (Thiemann *et al.*, 2007; Tynan and DeMaster, 1997; Young and Ferguson, 2014). For example, the abundance of polar cod, which were recovered from every adult stomach collected in 2012 (Table 2.6), has been shown to decrease in areas where average surface temperatures remain below -1.3 °C (Crawford and Jorgenson, 1996). Survival of larval fish has also been shown to increase during periods of low ice cover, particularly when sea surface temperatures remain within a relatively narrow thermal window (ca. -2–3.5°C; Fortier *et al*., 2006; Laurel *et al.*, 2018; Kono *et al.*, 2016). In contrast, Craig *et al.* (1982) reported that changes in ocean salinity, not temperature, were responsible for regulating cod abundance in the Beaufort Sea. Finally, Crawford and Jorgensen (1993) observed schools of polar cod were larger and more concentrated in areas with limited ice cover, but became progressively diffuse as the density of unconsolidated pack ice increased. Similar limiting factors have been identified for other common ringed seal prey, including, capelin (see Rose, 2005; Bluhm and Gradinger, 2008; Crook *et al.*, 2017) and *T. libellula.*

Ratios of muscle ^{15}N did not change in accordance with the timing of spring breakup or fall freeze-up, nor did they show significant interannual variability. However, similar to FA signatures, fluctuations in local prey availability may account for the slight year-to-year variation observed in adult seals. Hobson and Welch (1992) showed that ringed seals feeding on a diet consisting exclusively of amphipods and polar cod should have $\delta^{15}N$ values of 15.3‰ and 19.0‰, respectively. By extension, intermediate values ought to reflect a diet comprised of some combination of the two. Using samples collected in Lancaster Sound, they concluded that a mean $\delta^{15}N$ value of 17.3‰ likely signifies a diet consisting of approximately 54% polar cod, with the

remaining 46% attributable to various species of invertebrates (Hobson and Welch, 1992). Seals collected for the present study had mean $\delta^{15}N$ values ranging from 16.62 to 18.15‰ (Figure 2.5), suggesting the proportion of polar cod in their diets changed slightly from year-to-year. As noted above, these shifts in prey composition are likely responsible for the observed interannual variation in FA signatures. However, unlike FA signatures, which closely reflect the relative contribution of individual prey species to predator diets, $\delta^{15}N$ values alone provide a coarser resolution of foraging habits. Different prey may also share similar isotopic signatures if they occupy comparable ecological niches, particularly sympatric species, such as polar cod and capelin (McNicholl *et al.*, 2018). Thus, possible similarities in isotopic signatures among prey species, along with different sample sizes, may account for the discrepancy between the two diet estimation techniques in terms of their ability to detect a significant difference in seal diets between years.

Interannual variation in the timing of sea ice retreat and fall advance had a significant effect on the ratio of ^{13}C in the muscle tissue of adult ringed seals. Specifically, mean $\delta^{13}C$ values were lower during years in which the date of breakup occurred later in the spring/summer and were higher during years of early breakup. The opposite relationship was observed for ^{13}C values and freeze-up dates. Changes in $\delta^{13}C$ values are known to reflect shifts in the source of consumer diets, with benthic and inshore primary producers being enriched in ^{13}C relative to pelagic organisms. Ice algae, for example, is known to have higher mean $\delta^{13}C$ values than phytoplankton, which typically occurs in higher concentrations in open water (Arrigo *et al.*, 2014; Perrette *et al.*, 2011). Thus, my results indicate a decrease in the proportion of ringed seal diets originating in benthic and/or nearshore habitats during years in which breakup occurred later than average. These findings are contrary to expected results. They also differ from those

presented in previous studies, where periods of increased sea ice cover were associated with corresponding increases in $\delta^{13}C$ values for both ringed seals and polar bears (Young and Ferguson 2013; McKinney *et al.,* 2009). Although phytoplankton is most commonly associated with open water habitats, recent studies have documented large blooms occurring below thinning pack ice, particularly under areas with high concentrations of surface melt ponds (Arrigo *et al.*, 2014; Lowry *et al.,* 2014). Therefore, it is possible that seals may have consumed a diet derived from larger proportions of pelagic sources, such as phytoplankton, even during years of late breakup. Additional sample collection and further monitoring of both sea ice conditions and primary productivity may help clarify the relationship between ringed seal diets, prey abundance and sea ice phenology.

With the exception of $\delta^{13}C$ values, changes in the timing of breakup did not appear to directly regulate ringed seal diets over the five-year study period. However, elsewhere in the Canadian Arctic, ongoing reductions in annual sea ice concentration have been associated with long-term shifts in ringed seal prey composition. Analysis of stomach contents from seals collected in western Hudson Bay revealed a significant change in the proportion of fish species consumed over a period of approximately twenty years. Polar cod, ninespine stickleback (*Pungitius pungitius*) and sculpin were among the most important prey during the 1990s; however, in the 2000s, when the average date of sea ice breakup occurred several weeks earlier, ringed seal diets consisted primarily of capelin, sandlance and non-native rainbow smelt (Chambellant *et al.,* 2013). These long-term shifts in diet were accompanied by ongoing reductions in body condition and ovulation rates, and increased cortisol levels (Ferguson *et al.,* 2017). As annual sea ice conditions in the Gulf of Boothia continue to change, it is expected that

ringed seal prey composition may undergo similar shifts, ultimately leading to analogous declines in condition and vital rates.

The observed age-specific variation in fatty acid signatures and ratios of ^{15}N provides further evidence that ringed seals undergo an ontogenetic niche shift, likely transitioning from a diet dominated by crustaceans to a diet consisting of larger proportions of fish, such as polar cod. The FA signatures of adult seals exhibited inter-annual variation but did not change in relation to the date of spring breakup, suggesting changes in sea ice alone do not necessarily regulate ringed seal diets. Rather, a more complex suite of interacting extrinsic factors, such as ocean temperature, salinity and natural fluctuations in prey abundance, along with sea ice conditions likely influence ringed seal foraging habitats. My results do suggest that the origin of seal diets changed in relation to the timing of sea ice breakup, shifting from high mean $\delta^{13}C$ values during years of early breakup to lower values during years in which the date of breakup occurred later in the spring/summer. Unfortunately, FA and isotope signatures from ringed seal prey species were not available during this study; therefore, the methods used herein do not allow for the quantification of specific prey items or changes in their relative importance among age classes and/or over time. Further research, including the systematic collection and analysis of local prey, is needed to overcome these limitations. Additionally, monitoring of ringed seal body condition, recruitment and other vital rates, will help to identify changes in overall population health in the Gulf of Boothia and Prince Regent Inlet. Doing so will help to track and mitigate future climate-mediate changes in ringed seal abundance and the broader central Arctic food web.

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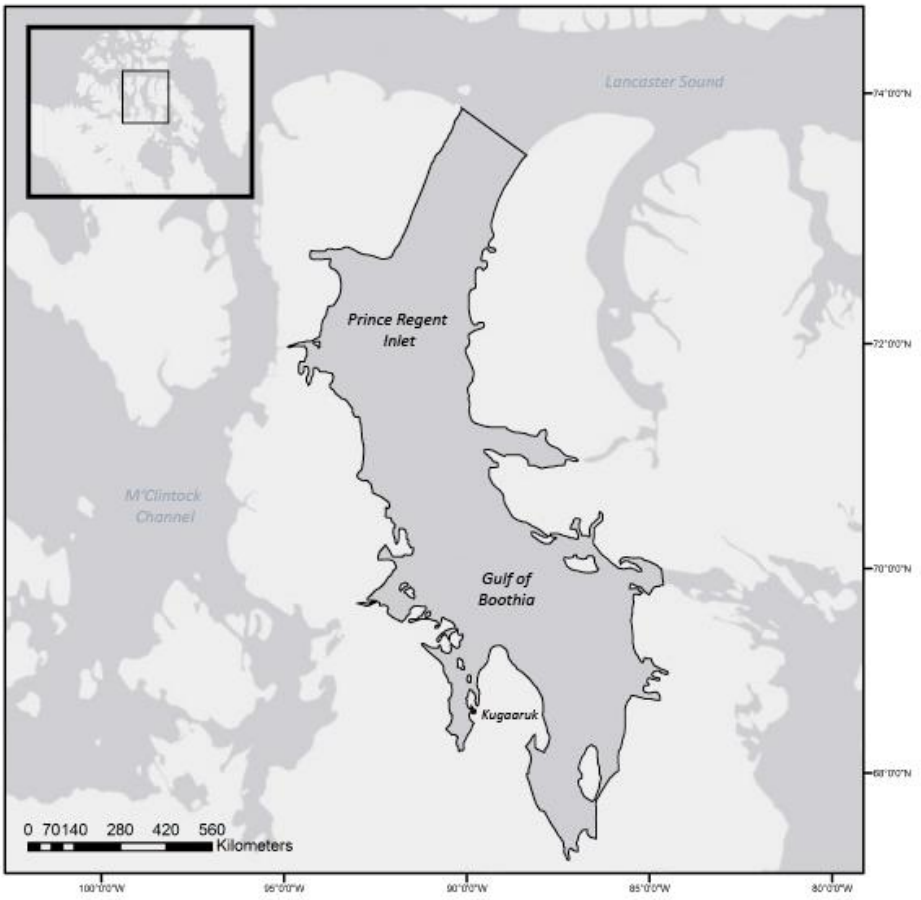

Figure 2.1. Study area extent (black outline) includes the Gulf of Boothia and Prince Regent Inlet, located adjacent to the hamlet of Kugaaruk, Nunavut. Inset shows location in Canada.

Table 2.1. The number of ringed seal samples from each age class (Adult, Juvenile, and Young of the Year [YOY]) included in the different diet analyses. Ringed seal stomachs were only collected in 2012.

Year	Fatty Acid Signature Analysis			Stable Isotope Analysis			Stomach Content Analysis		
	Adult	Juvenile	YOY	Adult	Juvenile	YOY	Adult	Juvenile	YOY
2012	10	3	2	6	1	1	12	2	3
2013	19	4	3	8	1	1	-	-	-
2014	24	4	14	8	1	1	-	-	-
2015	11	14	8	5	2	1	-	-	-
2016	37	2	5	8	2	0	-	-	-
Total		160			46			17	

Table 2.2. Fatty acid composition (mean mass % of total FAs ± SE) of blubber samples collected from ringed seals. Only those FAs that averaged > 0.1% across all age classes are presented. Bolded FAs are those used to assess differences in FA signatures among age classes; * denote the subset of FAs used to examine interannual difference in FA signatures among adult seals.

Fatty Acid	Adult *n* = 101	Juvenile *n* = 27	Young of the Year *n* = 32
Saturated Fat			
14:0	3.018 ± 0.064	3.514 ± 0.098	3.166 ± 0.077
15:0	0.146 ± 0.002	0.164 ± 0.003	0.170 ± 0.006
16:0 *	4.466 ± 0.155	5.421 ± 0.196	5.010 ± 0.187
18:0	0.626 ± 0.025	0.747 ± 0.042	0.580 ± 0.030
Monosaturated Fat			
14:1n-5	1.108 ± 0.050	0.889 ± 0.071	1.035 ± 0.003
14:1n-9	0.210 ± 0.005	0.171 ± 0.007	0.127 ± 0.007
16:1n-7 *	18.991 ± 0.290	21.516 ± 0.488	22.335 ± 0.471
16:1n-11	0.396 ± 0.013	0.325 ± 0.018	0.507 ± 0.018
16:1n-9	0.502 ± 0.018	0.385 ± 0.019	0.522 ± 0.018
17:1	0.165 ± 0.004	0.159 ± 0.010	0.223 ± 0.015
18:1n-5	0.699 ± 0.014	0.719 ± 0.030	0.776 ± 0.023
18:1n-7	4.085 ± 0.038	4.384 ± 0.072	5.277 ± 0.108
18:1n-9 *	12.863 ± 0.261	11.348 ± 0.345	14.881 ± 0.302
18:1n-11	3.998 ± 0.099	2.981 ± 0.135	3.732 ± 0.178
20:1n-9 *	9.901 ± 0.278	8.701 ± 0.577	6.679 ± 0.411
20:1n-7	0.943 ± 0.028	0.834 ± 0.054	0.672 ± 0.036
20:1n-11	2.001 ± 0.055	1.480 ± 0.070	1.416 ± 0.089
22:1n-11	2.147 ± 0.175	3.189 ± 0.430	1.055 ± 0.195
22:1n-7	0.123 ± 0.008	0.159 ± 0.018	0.075 ± 0.010
22:1n-9	0.767 ± 0.039	0.821 ± 0.084	0.403 ± 0.042
Polyunsaturated Fat			
16:2n-4	0.631 ± 0.019	0.600 ± 0.035	0.527 ± 0.022
16:3n-4	0.222 ± 0.006	0.239 ± 0.017	0.171 ± 0.009
16:4n-1	0.295 ± 0.018	0.367 ± 0.046	0.193 ± 0.024
18:2n-4	0.134 ± 0.002	0.146 ± 0.004	0.157 ± 0.004
18:2n-6 *	1.060 ± 0.017	1.111 ± 0.004	1.160± 0.029
18:3n-6	0.199 ± 0.007	0.225 ± 0.019	0.225 ± 0.013
18:3n-4	0.120 ± 0.002	0.115 ± 0.004	0.136 ± 0.003
18:3n-3	0.328 ± 0.007	0.304 ± 0.011	0.286 ± 0.008
18:4n-3	0.640 ± 0.022	0.707 ± 0.060	0.553 ± 0.030
18:4n-1	0.122 ± 0.003	0.143 ± 0.008	0.146 ± 0.004
20:2n-6	0.231 ± 0.006	0.204 ± 0.005	0.199 ± 0.006
20:3n-6	0.141 ± 0.003	0.120 ± 0.005	0.136 ± 0.004
20:4n-6	0.402 ± 0.009	0.407 ± 0.015	0.570 ± 0.023
20:4n-3	0296 ± 0.003	0.286 ± 0.007	0.264 ± 0.007
20:5n-3 *	8.653 ± 0.172	9.495 ± 0.455	8.714 ± 0.266
21:5n-3	0.410 ± 0.007	0.365 ± 0.015	0.347 ± 0.010
22:4n-6	0.121 ± 0.003	0.105 ± 0.004	0.116 ± 0.005
22:5n-3	6.676 ± 0.128	5.966 ± 0.210	5.862 ± 0.184
22:6n-3 *	10.202 ± 0.128	9.324 ± 0.209	9.622 ± 0.244

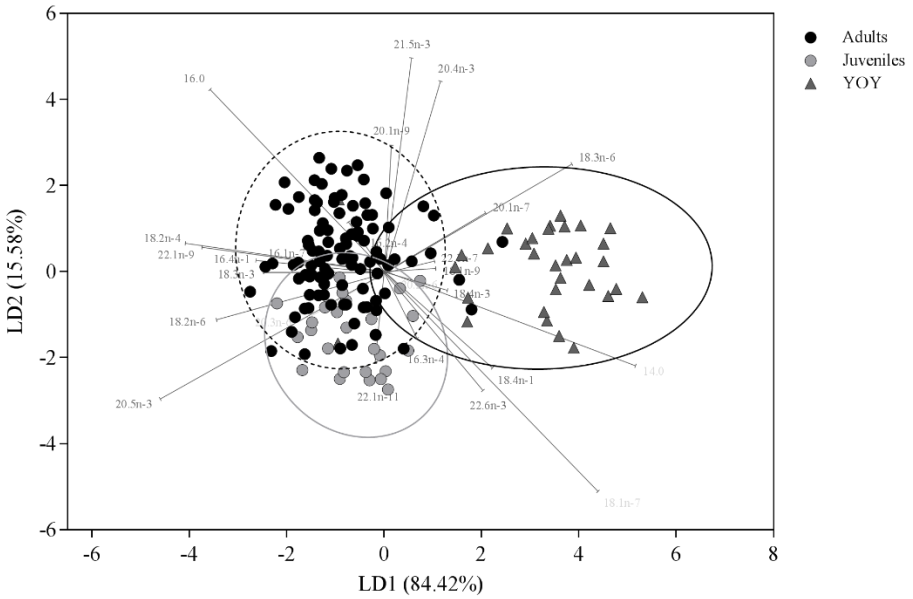

Figure 2.2. Discriminant function analysis of the most abundant and variable fatty acids from ringed seals of different age classes harvested in the Gulf of Boothia and Prince Regent Inlet, Nunavut. Ellipses represent the 95% confidence interval for each age class.

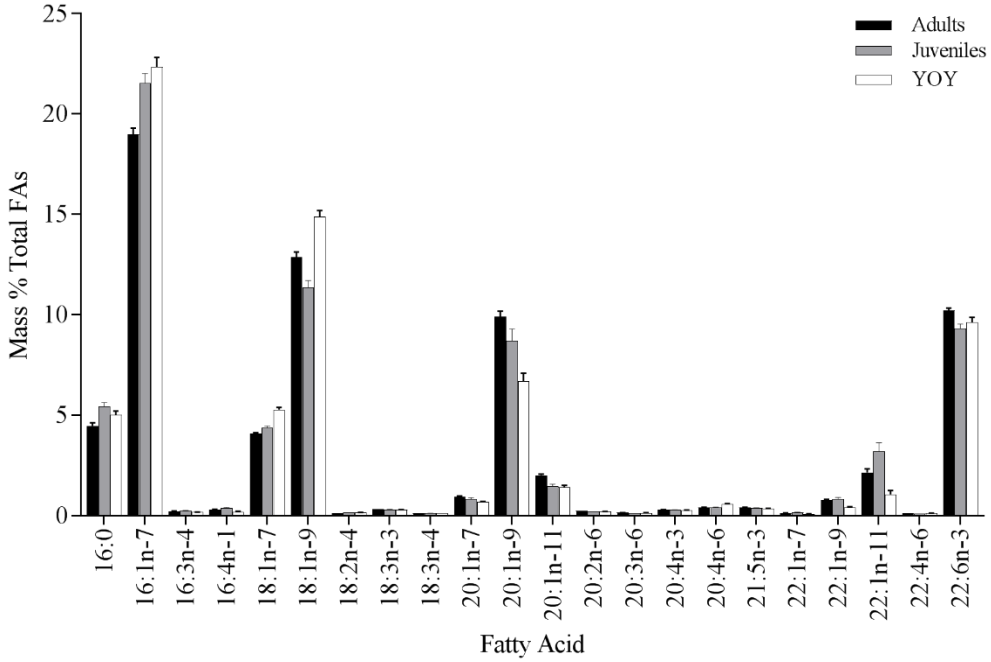

Figure 2.3. Twenty-two FAs (mass % of total) that showed significant variation (MANOVA: Wilk's $\lambda = 0.190$, $F_{(2, 157)} = 5.764$, $p = < 0.001$) among ringed seals of different age classes.

Table 2.3. *Post-hoc* pairwise comparisons of dietary FAs that differed between adults and juveniles (bold), adults and young of the year (*), and juveniles and young of the year (‡).

Fatty Acid	Adult *n* = 101	Juvenile *n* = 27	Young of the Year *n* = 32
16:0 * ‡	4.466 ± 0.015	5.421 ± 0.038	5.010 ± 0.033
16:1n-7 ‡	18.991 ± 0.029	21.516 ± 0.094	22.335 ± 0.083
18:1n-7 * ‡	4.085 ± 0.004	4.384 ± 0.014	5.277 ± 0.019
18:1n-9 ‡	12.863 ± 0.026	11.348 ± 0.067	14.881 ± 0.053
20:1n-9 *	9.901 ± 0.028	8.701 ± 0.111	6.679 ± 0.073
20:1n-7 *	0.943 ± 0.003	0.834 ± 0.010	0.672 ± 0.006
20:1n-11 *	2.001 ± 0.006	1.480 ± 0.014	1.416 ± 0.016
22:1n-11 * ‡	2.147 ± 0.017	3.189 ± 0.083	1.055 ± 0.035
22:1n-7 * ‡	0.123 ± 0.008	0.159 ± 0.004	0.075 ± 0.002
22:1n-9 * ‡	0.767 ± 0.004	0.821 ± 0.016	0.403 ± 0.008
16:3n-4	0.222 ± 0.001	0.239 ± 0.003	0.171 ± 0.002
16:4n-1	0.295 ± 0.002	0.367 ± 0.009	0.193 ± 0.004
18:2n-4 ‡	0.134 ± 0.000	0.146 ± 0.001	0.157 ± 0.001
18:3n-4 ‡	0.120 ± 0.000	0.115 ± 0.001	0.136 ± 0.000
18:3n-3	0.328 ± 0.001	0.304 ± 0.002	0.286 ± 0.001
20:2n-6	0.231 ± 0.001	0.204 ± 0.001	0.199 ± 0.001
20:3n-6 ‡	0.141 ± 0.000	0.120 ± 0.001	0.136 ± 0.001
20:4n-6 * ‡	0.402 ± 0.001	0.407 ± 0.003	0.570 ± 0.004
20:4n-3 *	0296 ± 0.000	0.286 ± 0.001	0.264 ± 0.001
21:5n-3 *	0.410 ± 0.001	0.365 ± 0.003	0.347 ± 0.002
22:4n-6 ‡	0.121 ± 0.00	0.105 ± 0.001	0.116 ± 0.001
22:6n-3 ‡	10.202 ± 0.013	9.324 ± 0.040	9.622 ± 0.043

Table 2.4. Mean (±SE) stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values in muscle tissue of ringed seals collected in Gulf of Boothia and Prince Regent Inlet, Nunavut.

Age Class	Sex	Sample Size	$\delta^{13}N$	$\delta^{15}C$
Adult	Females	18	16.990 ± 0.149	-19.239 ± 0.241
	Males	17	17.020 ± 0.128	-18.499 ± 0.201
Juvenile	Females	6	16.503 ± 0.366	-19.283 ± 0.169
	Males	1	17.100 ± 0.000	-18.796 ± 0.000
YOY	Females	2	18.004 ± 0.004	-19.116 ± 0.696
	Males	2	16.547 ± 0.280	-19.300 ± 0.900

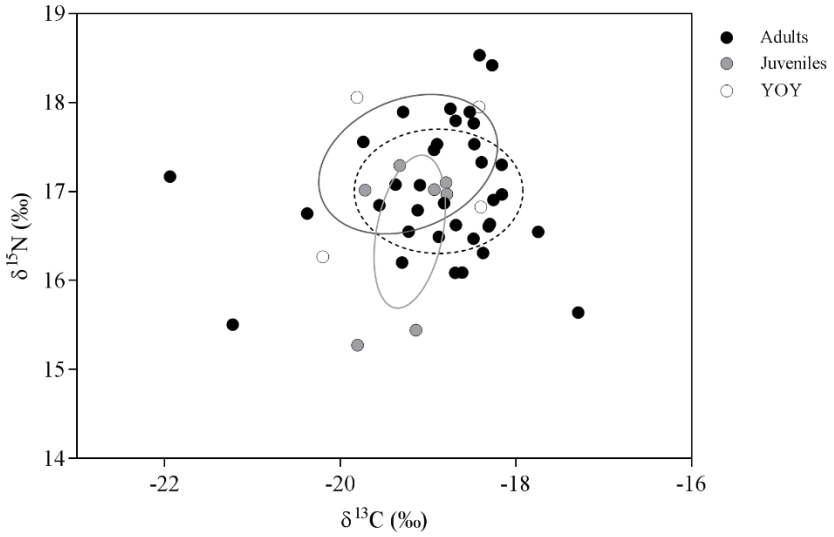

Figure 2.4. Biplot of muscle $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for ringed seals harvested in the Gulf of Boothia and Prince Regent Inlet between 2012 and 2016. Standard ellipse area with a correction for small sample sizes (SEAc) represent the core (95%) isotopic niche for each of the three age classes (Adults = dashed black line; Juveniles = solid grey line; YOY = solid black line).

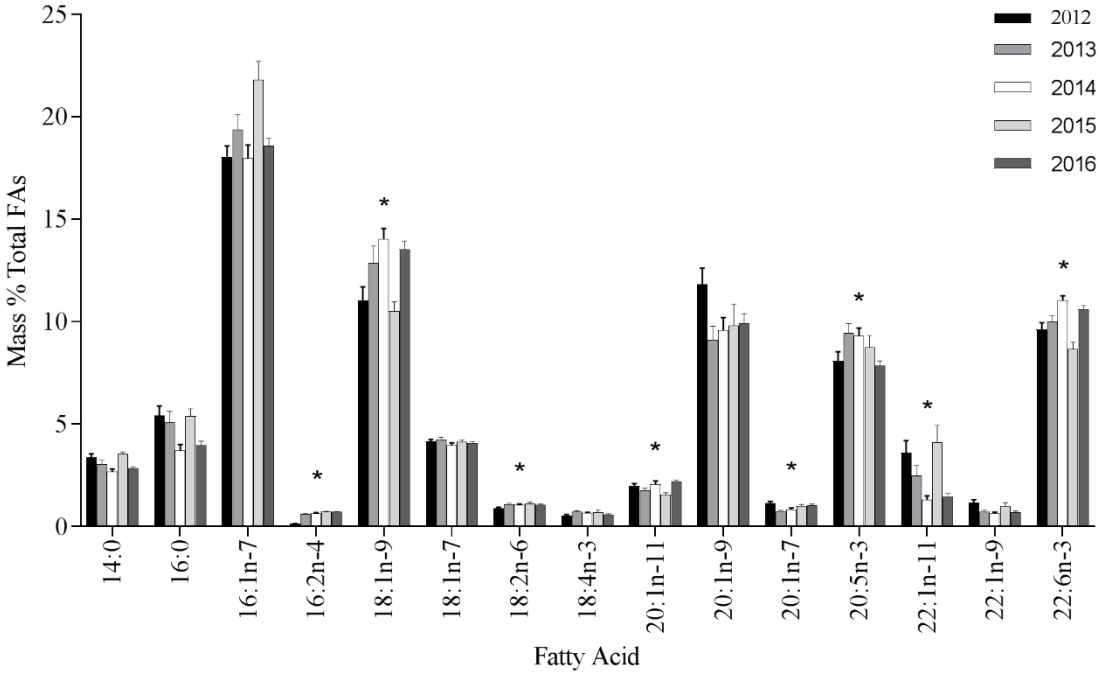

Figure 2.5. Fifteen FAs (mass % of total) evaluated in adult ringed seals over the course of the five-year study period. Asterisks (*) denote statistically significant differences in FAs that differed among years.

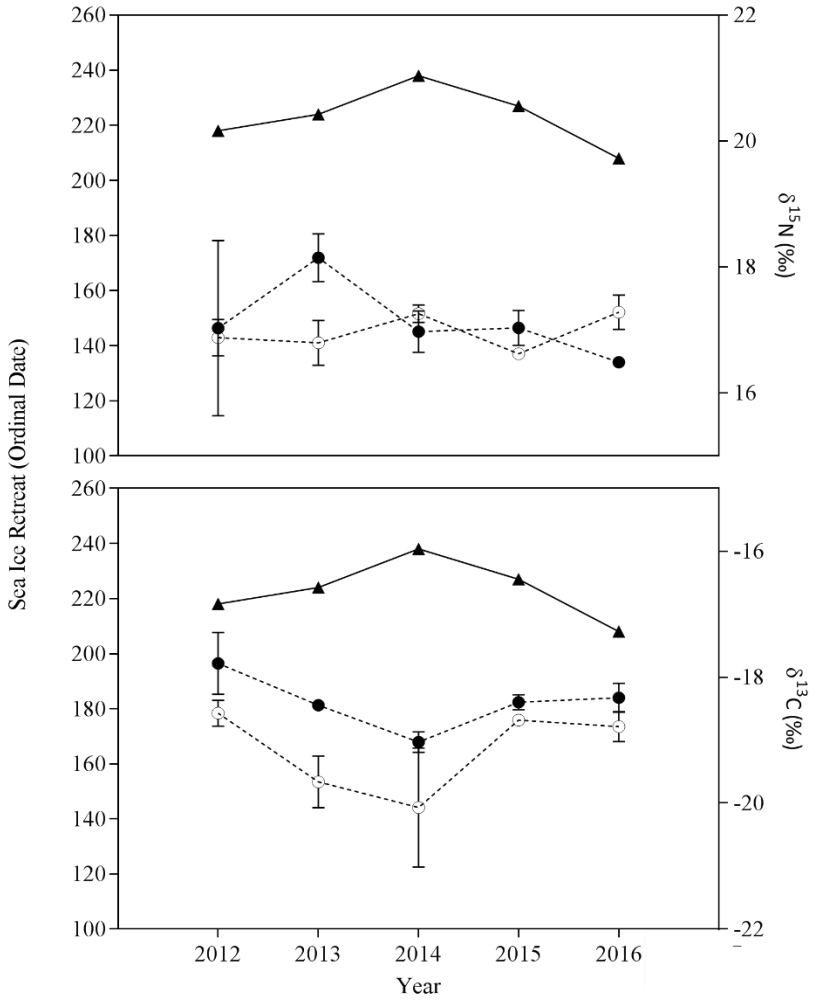

Figure 2.6. $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios (mean ± SE) of adult male (closed circles) and female (open circles) ringed seals in relation to the date of sea ice retreat (triangles) in the Gulf of Boothia and Prince Regent Inlet. Breakup date had a significant effect on $\delta^{13}C$ ratios ($F_{(2, 32)}$ = 9.084, p = < 0.001), but not on $\delta^{15}N$ ratios.

Table 2.5. Prey taxa collected from ringed seal stomachs collected in the Gulf of Boothia and Prince Regent Inlet during 2012; FO_i is the frequency of occurrence of each prey item, and N_i is the relative frequency of occurrence by number.

Prey	Adults		Juveniles		YOY		Total	
	FO_i	N_i	FO_i	N_i	FO_i	N_i	FO_i	N_i
Amphipoda								
Themisto libellulal	50.00	6.80					35.29	3.98
Gammarus wilkitzkii	41.67	1.92					29.41	1.12
Onisimus glacialis			33.33	0.16			5.88	0.05
Unidentified	16.67	0.40			33.33	0.33	17.65	0.33
Decapoda								
Lebbeus polaris	16.67	0.96					11.76	0.56
Mysidae								
Mysis oculata	50.00	38.18			100.00	98.45	52.94	50.57
Nematoda								
Unidentified	41.67	0.80					29.41	0.47
Fishes								
Boreogadus saida	100.00	48.50	100.00	98.72	66.67	0.41	94.12	41.14
Mallotus villosus	8.33	0.20					5.88	0.12
Sculpins (Cottidae)	41.67	1.88	50.00	1.28			35.29	1.26
Ammodytes sp.	8.33	0.04					5.88	0.02
Liparis sp.	25.00	0.32			66.67	0.65	29.41	0.37

Chapter III – Environmental Contaminants in Ringed Seals (*Pusa hispida*) from the Central Canadian Arctic: Evaluating Trends in Relation to Feeding and Sea Ice in the Gulf of Boothia

Introduction

Legacy contaminants, including persistent organochlorine pesticides (POPs) and trace elements such as mercury (Hg) and cadmium (Cd), have been studied throughout the circumpolar Arctic across both broad spatial and temporal scales (Muir *et al.*, 1999; Brown *et al.*, 2018; Rigét *et al.*, 2019). For many contaminants, clear geographic trends have emerged due to the ways in which they are transported and deposited into the Arctic environment (Braune *et al.*, 2005; Wagemann *et al.*, 1996; Brown *et al.*, 2018). Local and regional factors, such as environmental background concentrations of trace elements and anthropogenic sources of contamination, also influence their distribution (Muir *et al.*, 1999; Vorkamp and Muir, 2016). As the production and use of these contaminants have changed over the past several decades so too have the concentrations observed in terrestrial and marine biota. For instance, throughout the Canadian Arctic, concentrations of numerous POPs recorded in the fat stores of marine mammals have decreased significantly over the past forty years (Brown *et al.*, 2018; Rigét *et al.*, 2019). Mercury and numerous emerging chemicals, however, appear to be steadily increasing due to growing production and use in developing nations (Braune *et al.*, 2015; AMAP, 2017; Routti *et al.*, 2019).

While anthropogenic pollution is the predominant factor affecting contaminant concentrations in the Arctic, related environmental changes brought about by ongoing climate change have further altered chemical pathways and exposures (Macdonald *et al.*, 2000; McKinney *et al.*, 2015). This is particularly true for Arctic marine mammals wherein changes in

chemical concentrations have been directly linked to reductions in total sea ice extent, loss of thick multi-year ice, and changes in broad scale climate indices, such as the Arctic Oscillation and North Atlantic Oscillation (McKinney *et al.,* 2009; Gaden *et al.,* 2012; Cabrerizo *et al.,* 2018). Although the Arctic as a whole is warming at a rate more than twice the global average, the impacts of climate change have shown considerable regional variation (Serreze and Barry, 2011; Tynan and DeMaster, 1997). Long-term changes in sea ice in particular have varied considerably throughout the circumpolar Arctic (Stern and Laidre, 2016). Thus, continued monitoring of regional changes in contaminant concentrations is critical, as patterns of human use evolve and the effects of climate change alter patterns of transport and exposure in ways that are not yet fully understood.

Persistent organochlorine pollutants are a class of bioaccumulative and toxic chemicals that includes polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). These synthetic chemicals were produced in large quantities during the mid- to late-twentieth century for use in various industrial applications (PCBs) and as domestic pesticides (OCPs; Tanabe, 1988; Muir *et al.,* 1999). Due to their persistence and toxicity, restrictions on their production and use were enacted in the 1970s in most industrialized nations, followed by a global ban in 2004 (Muir *et al.,* 1999; Muir and Howard, 2006). Prior to these restrictions, PCBs and OCPs were readily transported to the Arctic via long-range atmospheric and oceanic currents, and secondarily through local sources such as riverine inputs (Muir *et al.,* 1999; Brown *et al.,* 2018). Trace elements derived from the combustion of fossil fuels and other anthropogenic sources continue to be transported to the Arctic; however, unlike POPs, they also occur naturally in sediments and other environmental reservoirs (Braune *et al.,* 2015; AMAP, 2017). Together, these chemicals are readily absorbed by living and dead organic matter in the Arctic marine

environment, where they both bioaccumulate and biomagnify in species occupying different trophic positions (Muir *et al.,* 1992). In several top marine predators they have been associated with a range of health impacts, including reproductive impairment, depressed immune function, and skeletal deformities (Vorkamp and Muir, 2016; Routti *et al.,* 2019).

Given the complex ways in which contaminants interact with the physical environment, one of the most efficient means of monitoring changes in their availability and persistence is through ongoing analysis of indicator species. Because of their high abundance, ubiquitous circumpolar distribution and central position within the Arctic marine food web, ringed seals (*Pusa hispida*) have frequently been used as a reliable bioindicator to monitor contaminant trends since the 1970s (Muir *et al.,* 1992). They are also an important food resource for Inuit and other northern Indigenous people; therefore, monitoring efforts have also been motivated by human-health considerations. Ringed seals are relatively small marine carnivores that feed opportunistically on a variety of crustaceans (amphipods, mysids, decapods, euphausiids) and small- to medium-sized fishes such as sandlance (*Ammodytes sp.*), sculpins (*Myoxocephalus sp.*), and polar cod (*Boreogadus saida*) (Young and Ferguson, 2014; Reeves, 1998; Holst, *et al.,* 2001). They are also routinely preyed upon by other marine predators, including polar bears (*Ursus maritimus)* and killer whales (*Orcinus orca*; Higdon *et al*., 2011; Pilfold *et al*., 2012). Thus, because of their trophic position, ringed seals facilitate the transfer of contaminants from primary and secondary consumers to apex predators, which have the highest concentrations of all species in Arctic marine food web.

Contaminant concentrations have been studied in ringed seals at numerous locations throughout the Canadian Arctic, particularly in the eastern and western Arctic where long-term monitoring dates back to the 1970s (Muir *et al.,* 1999). Recent efforts have also focused on seals

collected near several central Arctic locations, including Resolute Bay, Gjoa Haven and Arctic Bay; however, to date, no information is available for contaminant concentrations in ringed seals from the Gulf of Boothia and Prince Regent Inlet. Although little is known about ringed seal abundance in this region of the central Arctic, it supports the highest density of polar bears in the world (Taylor *et al.*, 2009; Hamilton and Derocher, 2019) and is among those areas most impacted by climate-driven sea ice changes. The timing of spring breakup in this region now occurs significantly earlier than it did forty years ago, retreating at a rate of approximately 8.6 days per decade^{-1}. Combined with later fall freeze-up, the number of ice-covered days has decreased by roughly 20 days per decade^{-1}, resulting in a considerably longer open water season (Stern and Laidre, 2016). Given the ongoing environmental changes and unknown levels of contaminants in ringed seals from this region, a community monitoring program was established in 2012 to collect and assess ringed seal tissues for concentrations of various legacy contaminants.

Concentrations of POPs and trace elements in Arctic marine mammals are influenced by a range of ecological and environmental factors. Specifically, due to their proclivity to bioaccumulate and biomagnify in marine food webs, organisms that feed on species occupying higher trophic levels tend to have elevated levels of many legacy contaminants (Hoekstra *et al.*, 2003; Atwell *et al.*, 2008). Also, positive phases of the Arctic Oscillation (AO) and North Atlantic Oscillation (NAO) have been linked with increased contaminant concentrations in Arctic species, as the prevailing winds associated with these periods facilitate the movement of chemical compounds northward from mid-latitude source locations (Houde *et al.*, 2019; Cabrerizo *et al.*, 2018). Higher proportions of first-year ice may also contribute to increased contaminant exposure because newly-formed sea ice melts more readily than multi-year ice,

releasing compounds that have been deposited within surface melt ponds on an annual basis (Pućko *et al.,* 2010). Thus, contaminant concentrations in ringed seals are expected to vary in accordance with these variables, likely increasing among those seals that feed on higher trophic level species, as well as in relation to positive phases of the AO and NAO indices and higher proportions of first-year ice. Here, I analyze concentrations of POPs and trace elements in ringed seals from the Gulf of Boothia and Prince Regent Inlet. Contaminants were assessed in relation to biological variables (age and sex), indices of foraging habits determined using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and several environmental variables, including duration of the open water season, sea ice type and concentration, and annual mean values of the Arctic Oscillation and North Atlantic Oscillation.

Methods

Sample Collection

Samples of blubber, muscle, and liver were collected from ringed seals harvested by Inuit hunters during the ongoing subsistence harvest in Kugaaruk, Nunavut, between 2012 and 2016. Seals were harvested from May to September of each year. Tissue samples were collected from a total of 188 ringed seals; however, only a portion of the samples were utilized for contaminant analysis (Table 3.1). All samples were initially frozen at -20°C and shipped to the Department of Fisheries and Ocean's Freshwater Institute in Winnipeg, MB, where they were subsequently stored at -35 °C prior to analysis. The sex of each seal was determined in the field at the time of sample collection. Ages were estimated by Matson Laboratory (Montana, USA) according to the methods outlined in Stirling *et al.* (1977) and Stewart *et al.* (1996). Briefly, the lower right canine tooth was removed, decalcified, longitudinally thin-sectioned and stained. The number of

annual growth layer groups (GLG) in the cementum of each tooth was then counted using a compound microscope and transmitted light.

Chemical Analyses & Quality Assurance

Concentrations of 90 PCB congeners, 15 OCPs (hexachlorobenzene, α-, β-, γ-hexachlorocyclohexane, heptachlor epoxide, oxychlordane, *trans*-, *cis*-chlordane, *trans*-, *cis*-nonachlor, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, dieldrin, and mirex), and percent lipid were measured in ringed seal blubber samples by ALSGlobal (Burlington, ON). Extraction and cleanup procedures followed those outlined in the US Environmental Protection Agency's Method 1699 (US Environmental Protection Agency, 2007a) with slight modifications. A 20 g aliquot of each sample was homogenized, spiked with $^{13}C_{12}$-PCB-133 and mixed with anhydrous sodium sulfate. The mixed phase samples were then extracted for approximately 24 hours using methylene chloride in a Soxhlet extractor with dichloromethane (DCM). The resultant extracts were cleaned using gel permeation chromatography (GPC) and reduced to a final volume of 1 mL in DCM. Prior to instrument analysis, 25 ^{13}C-labeled PCBs were added to the PCB extract for target analyte quantification and retention time reference. OCP extracts were mixed with 15 deuterated ^{13}C-labled OCPs for analyte quantification. Percent lipid was determined gravimetrically using a subsample of each extract. Samples were then analyzed using gas chromatography-low-resolution mass spectrometry (GC-LRMS) and gas chromatography-high-resolution mass spectrometry (GC-HRMS) for PCB congeners and OCPs, respectively. All data were recovery corrected for extraction and clean up losses relative to $^{13}C_{12}$-PCB-133.

Method detection limits (MLD) were determined for PCBs and OCPs using GC-MS instrument quantification at a signal to noise ratio of 2.5. For samples with overall detection frequencies >20%, individual analyte values below the MDL were replaced with 0.5*MDL.

Recoveries of the 25 ^{13}C-labeled PCBs ranged from 34% to 118%, while the 15 ^{13}C-labeled OCP standards ranged from 13% to 126%. Concentrations of PCBs were analyzed in blubber samples collected between 2012 and 2016, while OCPs concentrations were analyzed only in those samples collected between 2013 and 2016.

Ringed seal livers were analyzed for a suite of trace elements (arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), selenium (Se), lead (Pb), rubidium (Rd), and zinc (Zn)) at the National Lab for Environmental Testing (NLET; Burlington, ON). Samples weighing approximately 1 g were digested in nitric acid and hydrogen peroxide (8:1) in a high pressure microwave oven at 200°C for 15 minutes. The digested liver tissue was then analyzed directly using Inductively Coupled Plasma-Mass spectrometry (As, Cd, Cu, Pb, Se, Rb, and Zn), and cold vapor atomic absorption spectrometry (Hg). Total mercury in seal muscle was also determined by Direct Mercury Analyser (DMA 80, Milestone, Inc.) using the procedures outlined in the US EPA's method 7473 (US Environmental Protection Agency, 2007b).

All samples were analyzed in conjunction with reagent blanks and certified reference materials for quality assurance purposes; mean deviations from certified values were as follows: 7.3% (DOLT-4; dogfish liver), 14.8% (DORM-2; dogfish muscle), and 7.0% (TORT-2; lobster hepatopancreas). ALSGlobal and NLET are accredited by the Standards Council of Canada through the Canadian Environmental Laboratory program to the standard CAN-P-4D (ISO/IEC 17025:2005), and both labs have participated in the Northern Contaminants Program inter-lab quality assurance program.

Stable Isotopes

A subsample of muscle from each seal was analysed for stable isotopes of carbon and nitrogen at the University of Waterloo Environmental Isotope Lab in Waterloo, ON. Samples

were freeze-dried for approximately 48h, then crushed into a fine powder and homogenized. Lipids were extracted with 2:1 chloroform:methanol using a modified Bligh and Dyer (1959) procedure (Hobson *et al*., 1997; McMeans *et al*., 2009). Subsamples of the dried homogenate were then placed into tin capsules for analysis using a Thermo Finnigan-Deltaplus (Thermo Finnigan, USA) mass spectrometer in conjunction with a Costech elemental analyzer (Costech, USA). Analytic precision was determined to be ± 0.3‰ and 0.7‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Repeat analyses of internal laboratory standards calibrated against International Atomic Energy Agency Standards CH6 for carbon and N1 and N2 for nitrogen were conducted to ensure accuracy. Precision was assessed by analysing 15% of samples in duplicate, yielding a within-sample coefficient of variation of 0.014 for $\delta^{15}N$ and 0.017 for $\delta^{13}C$. Stable isotope ratios are expressed in parts per thousand (‰) using delta (δ) notation and are calculated using the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \cdot 1{,}000$$

where *X* is ^{15}N or ^{13}C, and *R* is equal to the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample and standard material. The recognized standards for stable carbon and nitrogen isotope analyses are Pee Dee belemnite limestone and atmospheric nitrogen, respectively.

Environmental Parameters

Weekly sea ice data for the Gulf of Boothia and Prince Regent Inlet were obtained from the Canadian Ice Service (https://iceweb1.cis.ec.gc.ca/CISWebApps/; accessed April, 2019). Mean sea ice concentration, as well as the extent of both old and first-year ice, were calculated for the study area from 2011 to 2016. Similar to the methods outlined in Etkin (1991) and Stirling *et al.* (1999), the annual break-up date for the region was defined as the day on which total sea ice concentration decreased and remained below 50% for at least one week. Annual

freeze-up date was defined as the day on which sea ice concentration increased and remained above 50%. Considering only weekly ice graphs were available, the dates of breakup and freeze-up were estimated by interpolating from the slope of the sea ice cline between the two weeks where concentrations were above and below 50%, respectively (Florko *et al.*, 2018). Duration of the annual open-water seasons was defined as the number of days between spring breakup and fall freeze-up.

Annual mean values of the North Atlantic Oscillation (NOA) and Arctic Oscillation (AO) indices were calculated from data provided by the National Oceanic and Atmospheric Administration (https://www.ncdc.noaa.gov/teleconnections/; accessed January 17, 2019). The NOA and AO influence large-scale weather patterns, including prevailing winds, precipitation and temperature regimes across much of the Arctic and into central Europe; both indices are known to influence the atmospheric transport of contaminants into the Polar Regions where they are then incorporated into terrestrial and marine food chains (Cabrerizo *et al.*, 2018; Houde *et al.*, 2019).

Statistical Analyses

Concentrations of PCBs and OCPs in blubber tissue are expressed in terms of lipid-weight, which was calculated by dividing wet-weight (ww) values by the proportion of lipid in each sample. Concentrations of trace elements in both ringed seal liver and muscle tissue are expressed as wet weight values. Prior to analysis, all contaminant concentrations were log-transformed in order to meet assumptions of normality and homogeneity of variance. The significance of statistical tests was assessed at $\alpha = 0.05$ and all analyses were conducted using R version 3.4.4 (R Core Team, 2013).

Previous studies have documented significant demographic (*i.e.* age and sex) variation in contaminant concentrations among ringed seals (Addison and Smith, 1974; Dehn *et al.*, 2005; Houde *et al.*, 2019). Therefore, in addition to testing for these relationships, subsequent analyses of organochlorine pesticides and trace elements controlled for the effects of both age and sex. Data were pooled for those compounds that did not differ significantly between sexes in order to increase sample sizes. Seals < 1 year of age were omitted from analyses in order to remove the effect of maternal contaminant transfers via mother's milk (Muir *et al.*, 2000).

In order to investigate how ringed seal foraging habits may influence contaminant burdens, relationships between chemical concentrations and log-transformed ratios of ^{13}C and ^{15}N were analyzed using simple linear regression. Partial Pearson correlation was used to test for relationships between contaminant concentrations (geometric means) and a suite of environmental parameters, including duration of the open water season, coverage of first-year ice, old ice and total sea ice throughout the study area, and both the AO and NAO indices. Annual geometric mean concentrations were compared with environmental variables from each respective year as well as the preceding year in order to test for possible lag effects in the influence of sea ice and climatic parameters on chemical concentrations in different seal tissues. Year was included as a covariate in each model to control for time trend effects.

Results

Values of select persistent organic compounds and trace elements are displayed in Figure 3.1. Individual POPs varied from an overall mean concentration of 0.33 ng/g l.w. (*trans*-chlordane) to 224.36 ng/g l.w. (ΣPCB), whereas hepatic trace elements ranged from 0.02 ng/g w.w. (Pb) to 11.66 ng/g w.w. (Zn). Concentrations of both OCPs and PCBs differed significantly among male and female ringed seals (OCPs: Wilk's $\lambda = 0.387$, $F_{(1,33)} = 5.453$, $p = << 0.001$;

PCBs: $F_{(1, 39)}$ = 22.310 , p = << 0.001) with males exhibiting consistently greater accumulation of most persistent compounds included in the present study. Overall, the same sex-related differences were not observed for concentrations of trace elements in seal liver tissue (Wilk's λ = 0.595, $F_{(1,38)}$ = 1.839, p = 0.101) or muscle mercury ($F_{(1, 39)}$ = 0.321 , p = 0.574); however, when examined individually, levels of hepatic copper, mercury, and selenium did differ among male and female seals. OCP and PCB concentrations were positively correlated with age in male seals, while concentrations in females remained relatively uniform across all ages (Figure 3.2).

Stable nitrogen (δ^{15}N) values ranged from 15.27 to 18.53‰, and despite the observed demographic differences in POP concentrations, ringed seals exhibited no significant variation in δ^{15}N between sexes ($F_{(1,39)}$ = 1.044, p = 0.313), ages ($F_{(1,39)}$ = 1.254, p = 0.270), or their interaction ($F_{(2,38)}$ = 1.008, p = 0.374). Values of stable carbon isotopes (δ^{13}C) ranged from -21.94 to -17.75‰ and differed among the sexes ($F_{(1,39)}$ = 8.203, p = 0.007); however, HCB was the only compound found to be correlated with δ^{13}C in seals of either sex (Table 3.2). In both male and female seals, δ^{15}N values were found to be correlated with several POPs and trace elements, including ΣPCB, *trans*-nonachlor (Table 3.2), arsenic, and muscle mercury (Table 3.3). Unlike concentrations of HCB, which appeared to decrease with increasing values of δ^{13}C, each of these compounds was positively correlated with δ^{15}N (Figure 3.3).

Sea ice conditions in the Gulf of Boothia and Prince Regent Inlet exhibited considerable interannual variation between 2011 and 2016; the duration of the open water season ranged from a low of 34 days in 2014 to a high of 117 days in 2011. First-year ice comprised the majority of sea ice in the region for the duration of the study (mean: 67.4%, SE: 1.3%), while old ice typically accounted for ca. 5% of total sea ice extent (mean: 4.7%, SE: 1.6%). The duration of the open water season during the year preceding sample collection was positively correlated with

muscle Hg concentration (r = 0.623, p = < 0.001; Table 3.4). The concentration of both old ice and total sea ice extent during the year prior to sample collection also influenced the accumulation of several POPs and trace elements in both male and female seals (Table 3.5). For example, old ice extent was negatively correlated with β-HCH and γ-HCH levels in male (r = - 0.744, p = 0.005) and female seals (r = - 0.214, p = 0.026), respectively, while total sea ice area was positively correlated with hexachlorobenzene concentrations in females (r = 0.360, p = 0.006). First-year ice extent during the year of sample collection was also correlated with concentrations of *trans*-nonachlor (r = - 0.998, p = 0.012) and ΣPCB (r = - 0.986, p = 0.012) for female seals (Figure 3.4). Levels of hepatic copper in both sexes were influenced by the total extent of sea ice during the year of sample collection (r = -0.516, p = 0.021; Figure 3.4) and the year prior (r = -0.958, p = 0.012). Lastly, arsenic was found to be positively correlated with the concentration of old ice during the year preceding sample collection (r = 0.976, p = 0.025).

Numerous relationships were found between the AO and NAO indices and concentrations of both POPs (Table 3.2) and trace elements in ringed seal tissues (Table 3.3). For male seals, the AO index during the year of sample collection was negatively correlated with ΣPCB, and both *trans*- and *cis*-chlordane (Figure 3.5), while a similar relationship was found for the AO index during the year prior to collection and β-BHC, ΣHCH, and heptachlor epoxide. Concentrations of γ-BHC, the only POP compound associated with the AO in females, were negatively correlated with index values during the year of sample collection. Concurrent values of the NOA were also negatively correlated with ΣHCH and heptachlor epoxide in male seals and positively correlated with γ-BHC in females (Figure 3.6). The two indices had similar associations with different trace elements, including selenium (Figure 3.5) and muscle mercury.

Discussion

Contaminant concentrations in ringed seals have been assessed across large spatial scales throughout the Canadian Arctic and have been shown to differ according to several biological and environmental factors (Vorkamp and Muir, 2016; Houde *et al.,* 2019; Braune *et al.,* 2015). The influence of certain variables, such as age and sex, are well-established and show little regional variation (Brown *et al.,* 2018; Muir *et al.,* 1999); however, site-specific associations between contaminants and sea ice conditions, as well as climatic patterns, suggest local differences in environmental conditions likely play a role in influencing contaminant pathways (McKinney *et al.,* 2015; Houde *et al.,* 2019; Cabrerizo *et al.,* 2018). Given this regional variability and the non-uniform way in which climate change is expected to affect Arctic ecosystems (Tynan and DeMaster, 1997), it is important to document contaminant concentrations in ringed seals from different locations to monitor attendant fine-scale changes in population health. This study is the first to document concentrations of legacy contaminants in ringed seals from the Gulf of Boothia and Prince Regent Inlet, Nunavut. Here, concentrations of numerous legacy contaminants measured in both liver and muscle tissue of ringed seals varied according to age and diet, as well as local sea ice conditions and broad scale climatic indices, closely reflecting concentrations observed in neighbouring regions of the Arctic.

Contaminant Concentrations in Gulf of Boothia Ringed Seals

Concentrations of environmental contaminants in marine mammals are known to vary spatially throughout the Arctic, often along longitudinal and/or latitudinal gradients due to different source locations and pathways of both transportation and deposition (Houde *et al.,* 2019; Macdonald *et al.,* 2000; Braune *et al.,* 2005). For instance, major organochlorine contaminants, including ∑PCB and ∑DDT, were found in lower concentrations in ringed seals from the Canadian Arctic than those sampled in East Greenland and Norway (Muir *et al.,* 2000;

Vorkamp and Muir, 2016). Accounting for these spatial trends, the observed concentrations of OCPs and PCBs recorded in the present study fell within the range of values expected for ringed seals in the Gulf of Boothia and Prince Regent Inlet. When compared with results from three different locations in the Canadian Arctic, annual geometric mean concentrations were most similar to those reported for seals harvested near Resolute Bay, located approximately 720 km northeast (Muir *et al.,* 2000). Comparisons with the remaining two locations, Sachs Harbour and Nain, located in the western and eastern Arctic, respectively, showed considerable variation among individual analytes; however, values remained within a 3.5-fold difference across all study sites.

Similar to organochlorine pesticides, trace elements have been shown to vary across longitudinal gradients throughout the Canadian Arctic. Brown *et al.* (2016), along with several previous studies, found mercury concentrations in muscle and liver tissue of ringed seals from the Beaufort Sea were approximately two- to three-fold higher than those in Hudson Bay and Labrador (Muir *et al.*, 1992; Wagemann and Muir, 1984*;* Braune *et al.*, 2015). Hepatic mercury concentrations recorded during the present study generally fell within the range of values expected for this region, as they were once again most comparable to those reported for Resolute Bay. Concentrations of muscle mercury, however, were most similar to values from Pond Inlet and Arctic Bay (Brown *et al.,* 2018). Interestingly, cadmium concentrations were considerably higher than those recorded for neighbouring regions, nearly double the values observed in Resolute Bay, and were instead closer to those reported for Eastern Arctic locations (Brown *et al.,* 2016). While differences in diet may partially account for these results, it is likely that environmental background concentrations also contribute to the higher values, as a similar trend was observed in polar bears from the same region (Brown *et al.*, 2018; Brown *et al.,* 2016; Routti

et al., 2011; Norstrom *et al.*, 1986). Concentrations of copper and zinc were most similar to those reported for seals from the Eastern Arctic (Northern Quebec, Arctic Bay, Resolute Bay), and values of lead and selenium, which were not recorded for western Arctic locations, differed from results presented herein by a factor of 2.3 and 1.2, respectively (Muir *et al.*, 1999).

Fewer studies have examined concentrations of the remaining trace elements (rubidium and arsenic) in ringed seal tissues, making detailed spatial comparisons difficult. However, Campbell *et al.* (2005) measured levels of rubidium in both freshwater and marine food webs at locations throughout Canada, including the North Water Polynya in Baffin Bay. The mean concentration recorded in ringed seals was 1.7 ± 0.3 μg/g ww, which is slightly lower than the mean value I observed (2.49 ± 0.09 μg/g ww). Similarly, ringed seals harvested at the south-western extent of Baffin Bay, near Pangnirtung, had hepatic arsenic concentrations of 0.48 ± 0.15 μg/g and 3.26 ± 0.81 μg/g (dry weight) (Kubota *et al.*, 2001; Ebisuda *et al.*, 2002), which are lower than the recorded mean concentration for seals in the Gulf of Boothia and Prince Regent Inlet. While a lack of data from other locations precluded more refined spatial comparisons, previous studies have examined these elements in liver tissue of polar bears from numerous sites throughout the circumpolar Arctic. Polar bears are the primary predator of ringed seals; therefore, for those contaminants that readily biomagnify within Arctic marine food chains, spatial patterns observed in polar bears likely reflect those in ringed seals (Muir *et al.*, 1992; AMAP, 1998; Campbell *et al.*, 2005) Further, due to region-specific differences in background concentrations of trace elements, bears and seals harvested from the same location ought to exhibit similar contaminant profiles and/or roughly consistent inter-species differences. Routti *et al.* (2011) showed that concentrations of rubidium were highest in polar bears from Western Hudson Bay and Davis Strait, yet overall, there were no geographical trends within or

across Canada, Greenland and Alaska. Arsenic concentrations were highest in the Western Arctic and followed similar spatial trends to mercury and selenium (*i.e.* increasing from east to west), which agreed with previous findings for both polar bears and ringed seals (Routti *et al.*, 2011; Rush *et al.*, 2008; Wagemann *et al.*, 1996).

The similarities between contaminant burdens observed in ringed seals collected as part of the present study and those from neighbouring regions, particularly Resolute Bay, are likely due to the ways in which legacy contaminants are transported and deposited throughout the Arctic. The Western Arctic Ocean, which lies upstream of the Canadian Arctic Archipelago, acts as a large reservoir for numerous legacy contaminants (Brown *et al.*, 2018; McKinney *et al.*, 2015; Rigét *et al.*, 2011). Ocean currents flow from the Western Arctic in an eastwardly direction, transiting the archipelago over a period of roughly three years, and eventually emptying into the north Atlantic (Brown *et al.*, 2018). As dissolved-phase contaminants move through the Canadian Arctic they undergo both sedimentation and absorption by organic carbon and animal tissues (Muir *et al.*, 1999). Therefore, while atmospheric deposition and environmental factors augment local availability, marine biota from neighbouring areas along this longitudinal gradient ought to share more similar contaminant profiles than those separated by greater distances.

Influence of Sex and Age on Contaminant Accumulation

Organochlorine concentrations varied significantly among ringed seals of different sexes with males exhibiting greater accumulation of ΣPCBs and most OCPs. These differences became increasingly pronounced in older individuals as contaminants continued to accumulate in males while concentrations in female seals remained relatively uniform across all ages. Results from previous studies have reported similar findings, suggesting post-parturient females pass many of

these lipophilic compounds on to their offspring via milk during the five to seven week nursing period (Addison and Smith, 1974). It has been estimated that lactating females may be able to transfer as much as 5-40% of their contaminant burden to an offspring within a period of 40 days (Hickie *et al.*, 2005). Males excrete small quantities of contaminants and their metabolites through urine and feces; however, the volume lost through these processes is insufficient to offset their accumulation over a lifetime.

Overall, concentrations of trace elements did not vary significantly between male and female ringed seals; although, examination of individual elements revealed female seals had higher but non-significant mean concentrations of hepatic copper, mercury and selenium. While previous studies have reported conflicting results (see Gaden *et al.* (2009) and Brown *et al.* (2016)), seals harvested near Ulukhaktok (formerly Holman), Northwest Territories and Barrow, Alaska exhibited significant sex-related differences in concentrations of several trace elements, including cadmium, silver and selenium (Dehn *et al.*, 2005). A similar trend was reported for ringed seals in Lake Saimaa, Finland where females had markedly higher levels of hepatic mercury (Lyytikäinen *et al.*, 2015). Sex-related variation in contaminant concentrations have been associated with dietary differences in several marine mammal species (Dehn *et al.*, 2005). However, the observed variation in trace element concentrations among male and female seals in the present study is unlikely related to diet, as both sexes had similar mean $\delta^{15}N$ values - a common metric used to assess trophic position. Rather, the differences in contaminant accumulation is likely due to higher feeding rates and energy requirements among females, specifically those that were pregnant or lactating. Females are estimated to have 30% higher energy needs than males, and a hyperactive metabolism during lactation may result in the transfer of Hg, and possibly other contaminants, from the blood directly into the liver

(Lyytikäinen *et al.*, 2015). While this is a plausible explanation for the observed sex-related differences, it is only speculative, as rates of pregnancy were not recorded for this study.

Influence of Foraging Habits

Due to their physical-chemical properties and persistence within the Arctic marine environment, many of the legacy contaminants considered in the present study biomagnify with increasing trophic level (Hoekstra *et al.*, 2003; Fisk *et al.*, 2001; Atwell *et al.*, 2008). Intraspecific differences in contaminant concentrations may also arise due to disparate foraging habits among individuals within the same population. For instance, walruses (*Odobenus rosmarus*) harvested in Inukjuak had significantly higher concentrations of several organochlorine contaminants than those from neighboring locations due to the greater proportion of ringed seals in their diet (Muir *et al.,* 1995). The influence of diet and trophic position on contaminant burdens is typically quantified using stable isotope analysis, as values of $\delta^{15}N$ increase in a relatively predictable manner from prey to predator (Kelly, 2000; Newsome *et al.*, 2010). Ratios of carbon isotopes on the other hand remain relatively stable across trophic levels, but can provide useful information on how foraging habitats (e.g. inshore vs offshore, benthic vs. pelagic) may impact exposure to different contaminants (DeNiro and Epstein, 1978; Dunton, 1989). Results from the present study show that after controlling for the effect of age, several contaminants were positively correlated with $\delta^{15}N$ values, while levels of HCB decreased with increasing values of $\delta^{13}C$.

Stable isotope patterns suggest that ringed seals feeding on a larger proportion of upper-trophic level organisms accumulated higher concentrations of select contaminants in their blubber and muscle tissues. Further, it appears that seals foraging on pelagic prey had higher concentrations of HCB than those feeding in more benthic environments, where values of $\delta^{13}C$

tend to be enriched. Ringed seals are known to consume a large variety of prey spanning several trophic levels (Holst *et al.,* 2001; Chambellant *et al.,* 2013; Fisk *et al.,* 2001). Examining stomach contents of seals harvested from the North Water Polynya, Holst *et al.* (2001) showed, collectively, their diet consisted of molluscs, mysids, several genera of amphipods, and both benthic and pelagic fishes. Due to their position in the Arctic marine food web, polar cod, which are the dominant food item for ringed seals, had the highest concentrations of OCs and muscle mercury when compared with other common prey (Fisk *et al.,* 2001; Gaden *et al.,* 2009). These contaminants are also known to bioaccumulate in organisms as they age, meaning ringed seals that consume a diet consisting of higher-trophic-level species, such as polar cod, and/or older, more contaminated individuals will be exposed to elevated levels of numerous legacy contaminants (Lockhart and Evans, 2000). Similar observations have been reported for other Arctic marine predators, including polar bears, Greenland sharks (*Somniosus microcephalus*), and narwhal (*Monodon Monoceros*) among others (Bentzen *et al.,* 2008; Fisk *et al.,* 2002; McKinney *et al.,* 2012).

The negative linear relationship between concentrations of HCB and $\delta^{13}C$ is likely a result of the lower hydrophobicity of this particular compound, which may render it less available to blooms of lipid-rich algae and, therefore, poorly transported to benthic organisms by sedimentation (Goutte *et al.,* 2013; Fahl and Kattner, 1993). Thus, as the results of the present study suggest, seals feeding on a greater proportion of benthic organisms ought to have lower values of HCB, while those feeding on a diet comprised of pelagic algae will exhibit higher concentrations.

Influence of Sea Ice

Sea ice concentration in the Gulf of Boothia and Prince Regent Inlet varied considerably over the five-year study period with thick first-year ice predominating throughout most of the region. The duration of the summer open-water season differed by as much as 83 days and was positively correlated with concentrations of muscle mercury. Gaden *et al.* (2009) reported a similar association for seals harvested near Ulukhaktok between 1973 and 2007 where a curvilinear relationship was found between muscle mercury and length of the ice-free season during the year preceding collection. Total mercury concentrations were highest during years following those with short (2 month) and long (5 month) ice-free seasons, whereas in the present study, mercury concentrations increased linearly with increasing lengths of the ice-free season during the previous year.

Ringed seals exhibit marked seasonal changes in foraging habits, alternating between periods of fasting during the winter and spring months and a brief period of hyperphagia during the late summer (Young and Ferguson, 2013). In addition to extending this period of intense foraging, longer open water seasons may also augment local productivity and prey assemblages, increasing the quantity of contaminants passed to upper-trophic-level predators such as ringed seals. For instance, longer ice-free seasons have been associated with increased productivity of phytoplankton, which form the base of the marine food web and indirectly feed numerous species, including *Themisto libellula* and polar cod (Renaut *et al.,* 2018). These species are important ringed seal prey and among the most contaminated lower-trophic consumers in terms of total mercury concentration (Loseto *et al.,* 2008; Gaden *et al.,* 2009). Therefore, the observed positive association between longer ice-free seasons and muscle mercury concentrations in ringed seals is likely due in part to increased productivity and consumption of contaminated prey. Because the majority of feeding occurs in late summer and fall, this increased accumulation

of mercury may not be evident until the following year. Finally, longer ice-free seasons may also allow for greater direct gas absorption of contaminants into ocean surface waters, rather than prolonged sequestration of particle phase compounds within ice and snow (Macdonald *et al.*, 2000; Muir *et al.,* 1999).

Multiple relationships were also found between contaminant concentrations and both sea ice type and extent throughout the study region. Old ice and total sea ice concentration during the year preceding sample collection were negatively correlated with isomers of HCH, copper and muscle mercury, and positively correlated with HCB and hepatic arsenic. First-year ice extent during the year of sample collection was negatively correlated with several groups of contaminants, including ∑PCB, *trans*-nonachlor and hepatic copper. Houde *et al.* (2019) reported similar relationships among POPs and sea ice concentrations, suggesting the specific type of ice, not total regional extent alone, may differentially influence the accumulation and availability of contaminants in Arctic marine environments.

Sea ice is a known reservoir for legacy contaminants, and depending on its stage of development, may sequester POPs and trace elements for different periods of time depending on the physical-chemical properties of individual compounds (Brown *et al.,* 2018; Pućko *et al.*, 2015; Grannas *et al.,* 2013). For instance, Pućko *et al.* (2010) showed that concentrations of α- and γ-HCH were higher in first-year ice due to the prevalence of melt water ponds containing brine, which serve as significant reservoirs for these particular OCPs. During the spring melt, these ponds often drain into surface waters where the dissolved-phase HCH isomers may be readily incorporated into the marine food web (Pućko *et al.*, 2010; 2015). Thicker multi-year ice sequesters contaminants for longer periods of time, whereas those contaminants that accumulate within first-year ice via atmospheric deposition or sediment resuspension are often released into

ocean surface waters within the same year (Grannas *et al.*, 2013; Macdonald *et al.*, 2000). Thus, the results presented herein suggest that the type of ice may differentially influence the accumulation and release of contaminants within Arctic marine environments; although, given the complexity of contaminant interactions within the cryosphere, further investigation is needed to fully elucidate these relationships.

Influence of Climactic Indices

Concentrations of several POPs and trace elements in ringed seal tissues collected from the Gulf of Boothia and Prince Regent Inlet were influenced by annual mean values of the Arctic Oscillation and/or North Atlantic Oscillation indices. The AO is a broad climate index characterized by surface atmospheric pressure patterns and winds circulating near 55° N latitude that track the strength of the polar vortex (Thompson and Wallace, 1998; Houde *et al.*, 2019). Positive phases of the AO are characterized by lower than average atmospheric pressure, which generally results in warmer and wetter winters across northern latitudes and movement of air from mid-latitude regions (Thompson *et al.*, 2000; Aanes *et al.*, 2002; Houde *et al.*, 2019). During negative phases, the AO index is characterized by high pressure, allowing cold Arctic air to move further south. For those contaminants associated with the AO index, most were found to be positively correlated with mean AO values from the year preceding collection. Concurrent AO values, on the other hand, were generally negatively correlated with contaminant concentrations in both ringed seal blubber and muscle tissue.

The NAO is characterized by shifts in atmospheric masses between the Arctic and subtropical Atlantic. Similar to the AO, it causes variation in surface pressure, which ultimately causes changes in mean wind speed and direction over the North Atlantic (Hurrell *et al.*, 2001). Although fewer significant correlations were observed for contaminant concentrations and the

NAO index, the same general trend was observed: NAO values during the year of sample collection were negatively correlated with contaminant concentrations. Correlations among data from the same year were too few (n = 2) to discern any meaningful trends. These results agree with those presented in previous studies, which reported positive associations between climate indices and contaminant concentrations in ringed seals harvested near Resolute Bay and Greenland (Houde *et al.,* 2019; Rigét *et al.*, 2013). Cabrerizo *et al.* (2018) also showed that concentrations of POPs were higher in Arctic char collected in Resolute Bay during years following positive AO and NAO phases. Given these observations, it is likely that positive phases of the AO and NAO help facilitate the transfer of more contaminants to the Arctic where they are deposited into surface waters and sea ice and ultimately incorporated into the marine food web (Houde *et al.,* 2019; Bustnes *et al.,* 2010; Rigét *et al.,* 2013).

Conclusion

Overall, concentrations of legacy contaminants in ringed seals from the Gulf of Boothia and Prince Regent Inlet were within the range of expected values given the known spatial trends of POPs and trace elements throughout the Canadian Arctic. Values were generally closest to those reported for seals from neighbouring Resolute Bay and other nearby locations where similar environmental conditions and proximity to contaminant reservoirs likely contribute to comparable exposure rates. Concentrations of POPs and several trace elements varied between sexes and increased as function age in male seals. These findings are consistent with those presented in previous studies and suggest that predators consuming higher proportions of adult seals (*e.g.* polar bears; see Pilfold *et al.,* 2012) are likely exposed to greater concentrations of legacy contaminants than those that routinely prey on younger age classes. Environmental variables, including sea ice type and relative concentration, as well as broad-scale climate indices

also influenced contaminant concentrations in ringed seals. While further investigation is required to fully understand these complex relationships, it is apparent that these variables, along with foraging habits and food web dynamics, interact to influence chemical pathways and exposure in ringed seals. Accordingly, continued monitoring of contaminants, including emerging compounds, in relation to environmental and ecological parameters will help to identify, and hopefully mitigate, future climate-mediated changes in the health of ringed seal populations and the broader Arctic marine community.

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Table 3.1. The number of samples collected from adult and juvenile ringed seals included in the different contaminant analyses. Young of the year (< 1yr) were omitted from analyses because of maternal transfer of contaminants via mother's milk. OCP data were not collected for 2012.

Year	*OCPs*		*PCBs*		*Trace Elements (Liver)*		*Hg (Muscle)*	
	M	F	M	F	M	F	M	F
2012	-	-	1	5	2	5	1	5
2013	2	7	2	7	1	6	2	7
2014	5	4	5	4	5	4	5	4
2015	5	2	5	2	5	2	5	2
2016	4	6	4	6	4	6	4	6
Total	35		41		40		41	

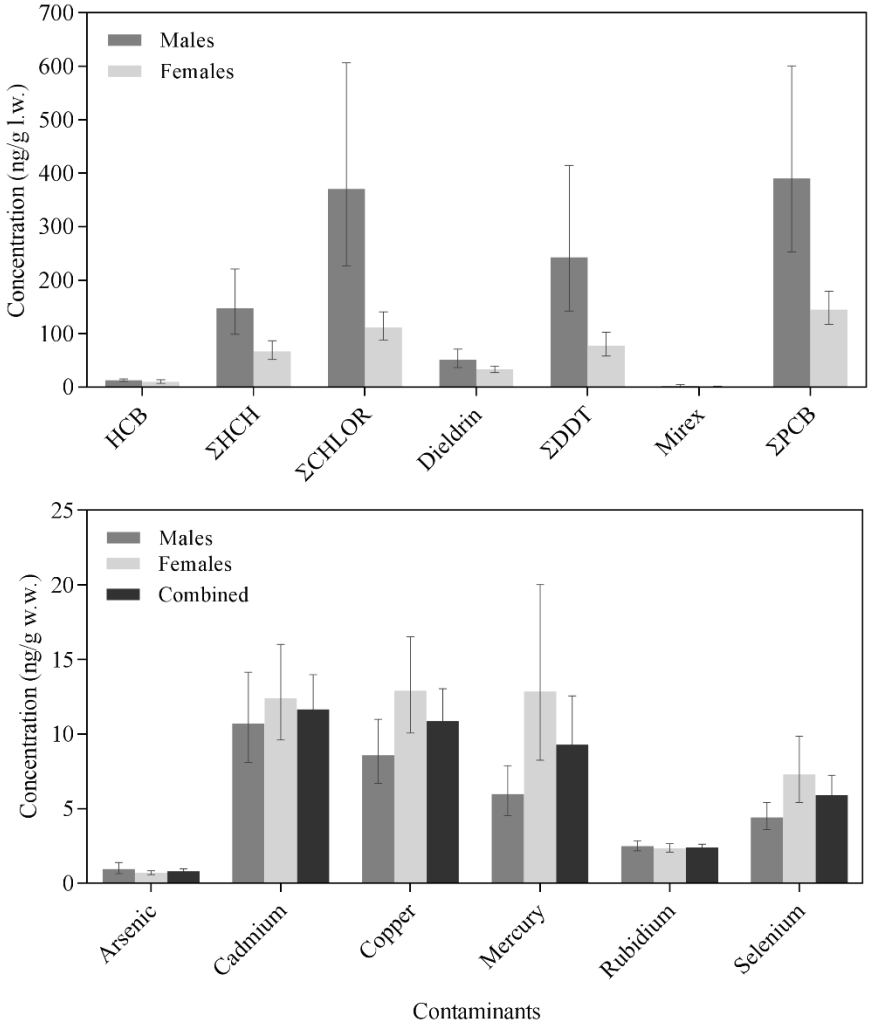

Figure 3.1. Concentrations (geometric mean and 95% CI) of select hepatic trace elements and persistent organic pollutants collected from blubber tissue of subadult (1-5 yr old) and adult (> 6 yr old) ringed seals in the Gulf of Boothia and Prince Regent Inlet, Nunavut. Values for lead (Pb; not shown) were: Male = 0.024 (0.015-0.038) ng/g w.w.; Female = 0.023 (0.016 – 0.031) ng/g w.w.; Combined = 0.023 (0.018 - 0.030) ng/g w.w.

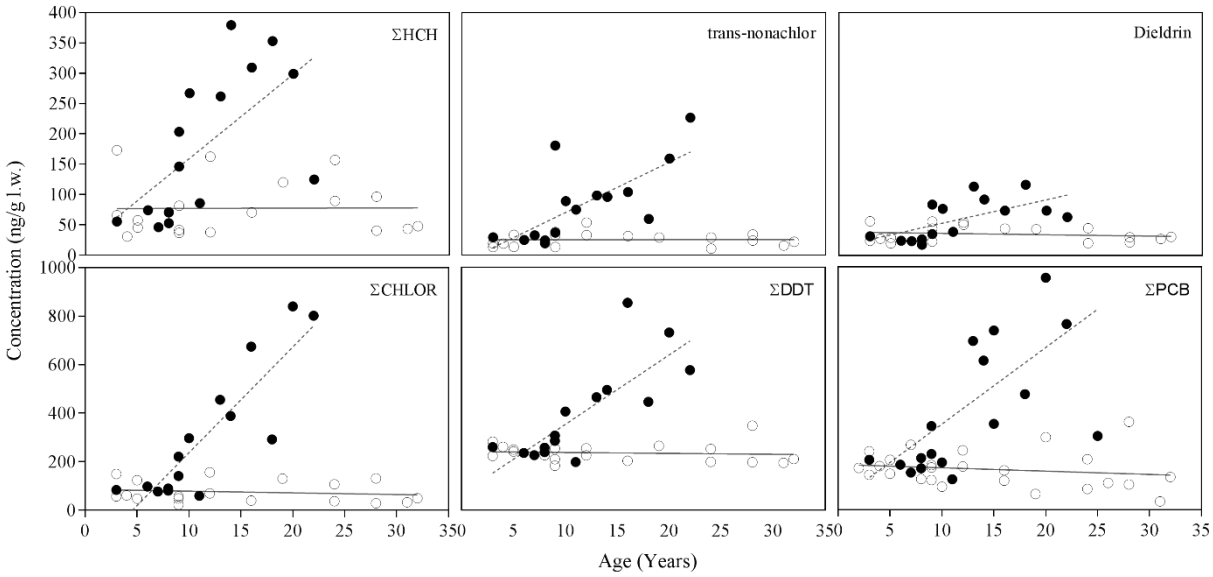

Figure 3.2. Linear regression showing the relationship between age and the accumulation of select persistent organic pollutants in blubber tissue among male (black circles) and female (open circles) ringed seals from the Gulf of Boothia and Prince Regent Inlet, NU.

Table 3.2. R-squared ($\delta^{13}C$ and $\delta^{15}N$) and partial Pearson correlations (Arctic Oscillation and North Atlantic Oscillation) adjusted for age and year between log concentrations of OCPs and PCBs in blubber of ringed seals harvested from the Gulf of Boothia and Prince Regent Inlet and both log-transformed stable isotope ratios and climatic indices.

Compound	Sex	Stable Isotopes		Arctic Oscillation		North American Oscillation	
		$\delta^{13}C$	$\delta^{15}N$	Current	Preceding	Current	Preceding
HCB	M	-0.23*					
	F						
α-HCH	M						
	F						-0.13**
β-HCH	M				0.64*		
	F						
γ-HCH	M						
	F				-0.98**		0.67*
ΣHCH	M				0.48*	-0.92***	
	F						
t-CHLOR	M						
	F						
c-CHLOR	M						
	F						
Oxy	M						
	F						
t-NON	M			-0.84*			
	F		0.65*				
c-NON	M			-0.69*			
	F						
Hept.	M				0.61*	-0.97**	
	F						
ΣCHLOR	M						
	F						
Dieldrin	M						
	F						
ΣDDT	M						
	F						
Mirex	M						
	F						
ΣPCB	M		0.41*	-0.47*			
	F						

HCB = hexachlorobenzene; HCH = hexachlorocyclohexane; CHLOR = chlordane; Oxy = oxychlordane; NON = nonachlor; Hept = heptachlor epoxide

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 3.3. R-squared ($\delta^{13}C$ and $\delta^{15}N$) and partial Pearson correlations (Arctic Oscillation and North Atlantic Oscillation) adjusted for age and year between log concentrations of trace elements in liver and muscle tissue of ringed seals harvested from the Gulf of Boothia and Prince Regent Inlet and both log-transformed stable isotope ratios and climatic indices.

Compound	Stable Isotopes		Arctic Oscillation		North American Oscillation	
	$\delta^{13}C$	$\delta^{15}N$	Current	Preceding	Current	Preceding
Liver						
Arsenic		0.414*				
Cadmium						
Copper						
Lead						
Mercury						
Rubidium						
Selenium			-0.918*			
Muscle						
Mercury		0.427**		0.844*	-0.356*	

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

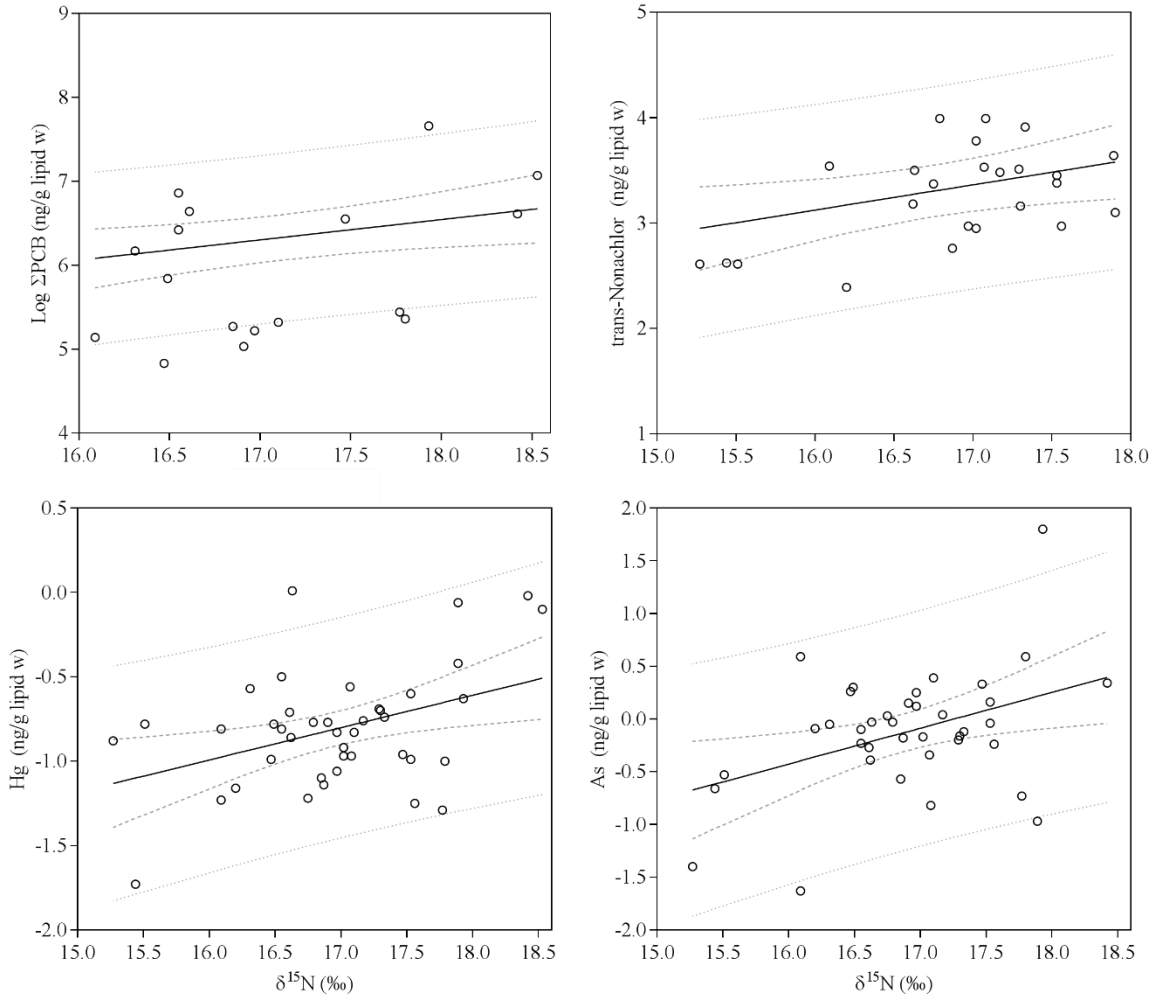

Figure 3.3. Observed (open circles) and predicted values (solid line) of log ΣPCB, *trans*-nonachlor, mercury (muscle) and arsenic for normalized male ringed seals based on regression equation where age was set at a constant mean value of 11.89. Larger dashed line is the 95% confidence interval, and the smaller dashed line represents the prediction interval.

Table 3.4. Partial Pearson correlations adjusted for age and year between log concentrations of trace elements in the liver and muscle of ringed seals harvested from the Gulf of Boothia and Prince Regent Inlet and duration of the open water season (Open Water), and concentration of first year, old and total sea ice extent during the year of sampling (Cur./Current Year) and the year prior (Prev./Previous Year).

Compound	Open Water		Current Year			Previous Year		
	Cur.	Prev.	Total	1st yr	Old	Total	1st yr	Old
Liver								
Arsenic								0.98*
Cadmium								
Copper			- 0.52 *			- 0.96 *		
Lead								
Mercury								
Rubidium								
Selenium								
Muscle								
Mercury		0.62***						

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 3.5. Partial Pearson correlations adjusted for age and year between log concentrations of OCPs and PCBs in blubber of ringed seals harvested from the Gulf of Boothia and Prince Regent Inlet and duration of the open water season (Open Water), and concentration of first year, old and total sea ice extent during the year of sampling (Cur./Current Year) and the year prior (Prev./Previous Year).

Compound	Sex	Open Water		Current Year			Previous Year		
		Cur.	Prev.	Total	1st yr	Old	Total	1st yr	Old
HCB	M						0.36**		
	F								
α-HCH	M								
	F								
β-HCH	M					- 0.74**			
	F								
γ-HCH	M								
	F					- 0.21*			
ΣHCH	M								
	F								
t-CHLOR	M								
	F								
c-CHLOR	M								
	F								
Oxy	M								
	F								
t-NON	M			-0.99**					
	F								
c-NON	M								
	F								
Hept.	M								
	F								
ΣCHLOR	M								
	F								
Dieldrin	M								
	F								
ΣDDT	M								
	F								
Mirex	M								
	F								
ΣPCB	M								
	F			-0.99*					

HCB = hexachlorobenzene; HCH = hexachlorocyclohexane; CHLOR = chlordane; Oxy = oxychlordane; NON = nonachlor; Hept = heptachlor epoxide

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

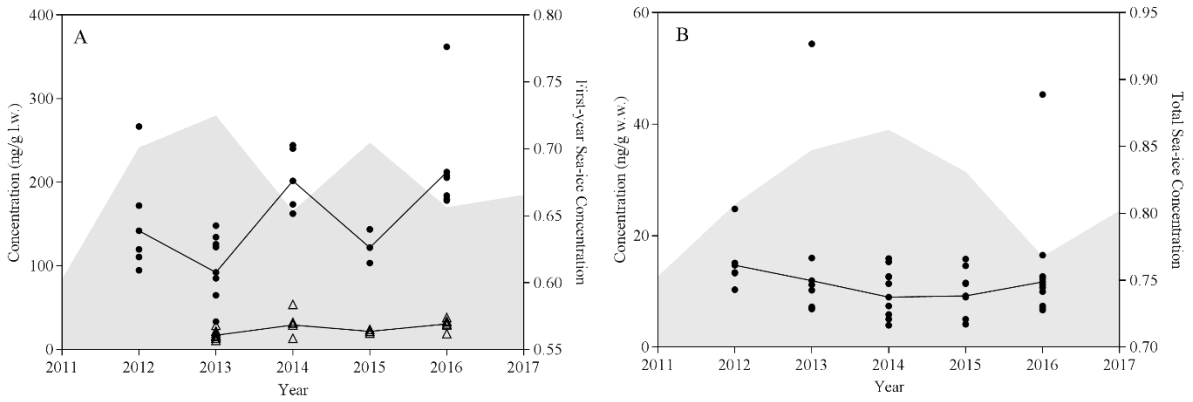

Figure 3.4. Concentrations of (A) ΣPCB (black circles) and *trans*-nonachlor (open triangles), and (B) hepatic Se in relation to mean values of first-year and total sea ice extent during the year of sample collection. Solid lines represent annual geometric mean.

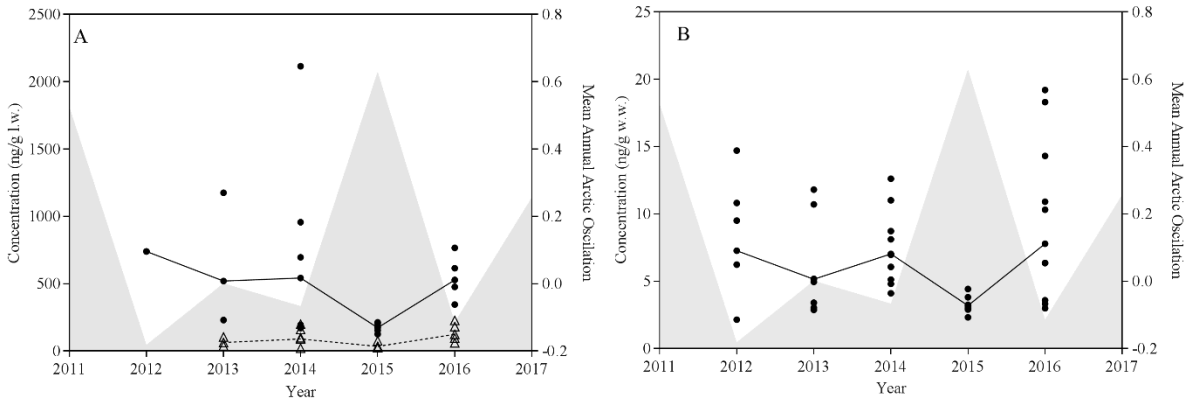

Figure 3.5. Concentrations of (A) ΣPCB (black circles) and *trans*-nonachlor (open triangles), and (B) hepatic Cu in relation to mean values of the Arctic Oscillation Index during the year of sample collection. Solid lines represent annual geometric mean.

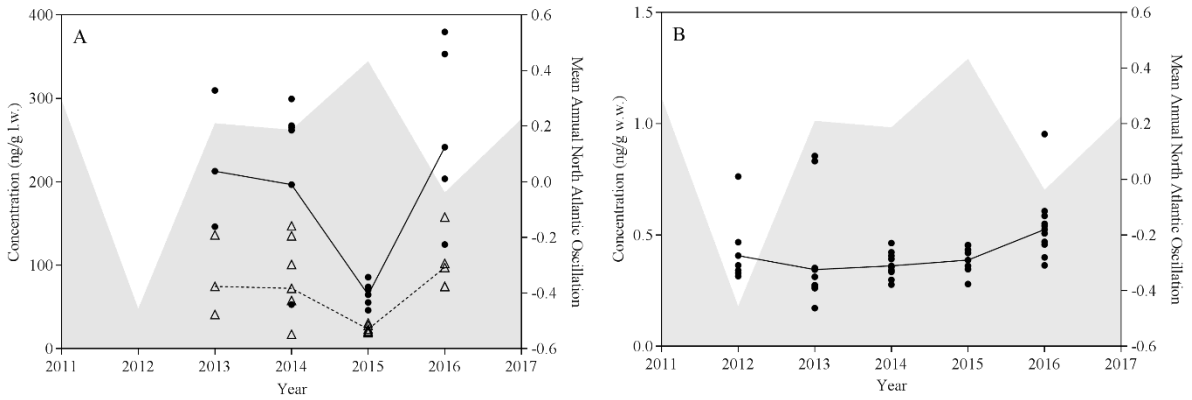

Figure 3.6. Concentrations of (A) ΣHCH (black circles) and heptachlor epoxide (open triangles), and (B) muscle Hg in relation to mean values of the North Atlantic Oscillation Index during the year of sample collection. Solid lines represent annual geometric mean.

Chapter IV – General Conclusion

Summary

Ringed seals are thought to play an integral role in the community structure of Arctic marine ecosystems. They occupy an important position within circumpolar food webs, serving as both predators of primary and secondary consumers and prey for apex predators, including polar bears (Holst *et al*., 2001; Ramsay and Stirling, 1988). Inuit throughout the Canadian Arctic also routinely hunt ringed seals for subsistence purposes and rely on them as a source of food and income (Reeves, 1998; Chambellant *et al.*, 2012). Despite their ecological and socio-cultural importance, relatively little is known about the ways in which seal abundance in the central Canadian Arctic is influenced by factors such as sea ice dynamics, prey availability and contaminant burdens. In this thesis, I examined the foraging habits of ringed seals in the Gulf of Boothia and Prince Regent Inlet over a five-year period in relation to both demographic and environmental factors. I also examined the influence of sea ice phenology and broad-scale climate indices on the concentration of legacy contaminants in ringed seal tissues, and compared observed values with those reported from other locations. Overall, the results of my thesis provide novel insights into the ecology and population health of ringed seals in the Gulf of Boothia and Prince Regent, and establish baseline information against which future changes can be measured as climate change continues to affect marine ecosystems throughout the Arctic.

Previous studies have documented age-related differences in the foraging habits of ringed seals. (Thiemann et al., 2007; Yurkowski et al., 2016.). My results support these findings, as both FA signatures and ratios of stable nitrogen isotopes differed among age classes, particularly between adult and juvenile seals. While I was unable to quantitatively assess differences in the

composition of their diets, adults did have higher mean $\delta^{15}N$ values, suggesting they consume a greater proportion of species occupying higher trophic levels. The diets of ringed seal pups closely reflected those of adults likely because both FAs and stable isotopes are passed from mother to young via milk during the two-month period following parturition (Hobson *et al.,* 1997; Holst *et al.,* 2001). Overall, FA signatures of adult seals exhibited significant interannual variation but did not differ in accordance with changes in the annual dates of sea ice breakup or freeze-up. Rather, the observed year-to-year variation is likely attributable to natural fluctuations in prey abundance and distribution caused by complex interactions between several extrinsic factors. Sea ice phenology did influence ratios of muscle ^{13}C, suggesting seals may have consumed a diet derived from larger proportions of pelagic sources, such as phytoplankton, during years of late breakup. Additional sample collection and further monitoring are required to further elucidate these relationships.

Given known spatial trends in the distribution of environmental contaminants throughout the Arctic, concentrations of persistent organic pollutants in the tissues of ringed seals from the Gulf of Boothia and Prince Regent Inlet generally fell within the range of expected values. Similar to previous studies, I found concentrations of certain legacy contaminants were influenced by both intrinsic and extrinsic factors, including age and sex, as well as sea ice phenology and broad-scale climatic patterns (Dehn *et al.,* 2005; Houde *et al.,* 2019). Specifically, concentrations of POPs and several trace elements varied between sexes and increased as a function of age in male seals, but remained relatively uniform in females. Changes in annual dates of breakup and freeze-up, and shifts in the AO and NAO indices differentially affected observed concentrations of several compounds. While further investigation is required to fully understand these complex

relationships, it is apparent that these variables, along with foraging habits, interact to influence chemical pathways and exposure in ringed seals.

Conservation Implications

Ringed seals are inextricably reliant on sea ice, as it provides critical habitat for several essential life history events, including mating and parturition. Consequently, increasing annual temperatures and attendant reductions in sea ice extent pose direct threats to ringed seal populations via habitat degradation. Ongoing climate change also poses several indirect threats, as shifts in environmental conditions have been associated with changes in prey distributions and contaminant exposure (Chambellant *et al.*, 2013; Gaden *et al.*, 2012; Houde *et al.*, 2019). For instance, during the 1990s, ringed seals in Hudson Bay routinely preyed on Arctic cod; however, by 2005, this common forage fish was absent from seal diets and had been largely replaced by capelin, a common temperate species (Chambellant *et al.*, 2013). This shift in prey composition coincided with decreases in seal density, recruitment and body condition, as well as increased physiological stress (Ferguson *et al.*, 2017).

Climate change may also alter the ways in which pollutants are transported, released and ultimately deposited in the Arctic. For example, warmer temperatures and longer ice-free seasons may allow for greater direct absorption of gas-phase contaminants into ocean surface waters where they can be readily incorporated into the marine food chain (Macdonald *et al.*, 2000). Coupled with the emergence of new environmental pollutants, these ongoing changes may result in higher rates of contaminant exposure and associated morbidity in ringed seals.

To my knowledge, this is the first study to document the foraging habits and contaminant burdens of ringed seals in this region of the central Canadian Arctic. Therefore, continued monitoring efforts are important to assess how the observed trends may change over time and in

relation to ongoing climate change. These efforts will help wildlife managers and other stakeholders identify and possibly mitigate future changes in ringed seal population health that may arise due to shifts in diet and/or increased contaminant exposure.

Future Research

I used fatty acid and stable isotope signatures to qualitatively assess the foraging habits of ringed seals in relation to both intrinsic (sex and age) and extrinsic factors (sea ice phenology). Results showed that seal diets differed among age classes and throughout the duration of the study. However, in the absence of complimentary dietary signatures from potential prey species, these analyses reveal little about the actual composition of the seals' diet (Budge *et al.*, 2006). While stomach content analysis did provide some insight into the types of species consumed, there were not enough samples available to make meaningful inferences about intraspecific differences or changes in relation to environmental variables. With sufficient FA data collected from potential prey, quantitative estimates of a predator's diet may be determined using a technique known as quantitative fatty acid signature analyses (QFASA). This technique allows one to model the proportional contributions of different prey species to a consumer's diet, and by extension, monitor how they may differ in accordance with a range of variables (Iverson *et al.*, 2004; Thiemann, 2009; Budge *et al.*, 2006). Similarly, isotopic signatures from potential prey can be incorporated into stable isotope mixing models to determine the composition of a consumer's assimilated die (Phillips *et al.*, 2014). Both techniques have been used to study the foraging habits of marine mammals throughout the circumpolar Arctic and could be used to obtain a more detailed understanding of ringed seal diets in the Gulf of Boothia and Prince Regent Inlet.

Contaminant concentrations in ringed seal tissues were examined in relation to demographic and environmental variables. General spatial comparisons were also made with results obtained in other regions of the Canadian Arctic where, for some locations, the monitoring of contaminants in ringed seals spans several decades. Unfortunately, the duration of the present study was too short to discern any meaningful temporal trends. Monitoring programs often need approximately 10-20 years of data in order to detect a 5% annual change in median contaminant concentrations with a sufficient degree of statistical power – a threshold used by the Arctic Monitoring and Assessment Program for time-trend analyses (AMAP, 2015). Therefore, the continuation of ringed seal sample collection in the Gulf of Boothia should be considered a priority, as it will eventually allow for the assessment of long-term trends in seal contaminant exposure and over-all population health. Future research should also consider a wider range of contaminants, particularly those that have been identified as 'emerging compounds,' as well as those that have only recently been discovered in other Arctic marine mammals (see Liu *et al.*, 2918). The effects of these contaminants are generally less well understood and likely pose a greater long-term risk to the health of Arctic biota.

Lastly, very little is known about the distribution and abundance of ringed seals in this region of the Canadian Arctic. Previous research conducted in Hudson Bay documented a gradual decline in seal density between 1995 and 2013, a trend that was accompanied by shifts in prey abundance, loss of sea ice habitat and increased physiological stress (Ferguson *et al.*, 2017; Young *et al.*, 2015). Another study examining seal movements showed that sea ice phenology affected the distance adult and juvenile seals travelled, likely because of its influence on prey distribution and habitat quality (Yurkowski *et al.*, 2016). Combined with monitoring efforts focused on foraging habits, physiology and contaminant exposure, a more thorough

understanding of these factors will enable stakeholders to better address projected climate-mediated declines in their abundance and possible ramifications on the broader marine food web.

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